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THE

AMERICAN JOURNAL
OF THE MEDICAL SCIENCES

DECEMBER, 1941

ORIGINAL ARTICLES.

ACETYLPHENYLHYDRAZINE ANEMIA. 1. THE MECHANISM OF ERYTHROCYTE DESTRUCTION AND REGENERATION.

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This paper deals with preliminary studies of erythrocyte regeneration in acetylphenylhydrazine anemia—a condition in which many products of red blood cell disintegration remain within the body economy. Detailed hematologic observations of red blood cells made at short intervals of time and pathologic examination of tissues selected at critical points have proved useful in improving the understanding of physiologic agencies active in the destruction of damaged erythrocytes and in the reconstruction of new red blood cells. The observations described may to some extent be helpful in understanding types of anemia in which the organism does not need exogenous material to rebuild the destroyed erythrocytes.

Methods. Different types of dogs have been used in these experiments. Normal dogs (Nos. 39–1, 39–57 and 40–213); splenectomized dogs (Nos. 39–194, 40–115 and 40–152); dogs with a gall-bladder renal fistula (Nos. 37–137 and 40–248); dogs made anemic by bleeding, and therefore with depleted iron reserves (Nos. 39–26 and 39–299); as well as a dog with a Pavlov pouch (No. 39–317). Normal rabbits (Nos. 3 and 4) were also observed.

In all of these 13 animals acetylphenylhydrazine (pyrodin*) was used for red blood cell breakdown. The injections were made subcutaneously beneath the skin of the abdomen. Pyrodin was dissolved in 10 to 20 cc. of hot saline just prior to the administration.

Hematologic methods used required only small amounts of blood. Generally 1 or 2 cc., rarely 5 cc., were taken from the jugular vein for each sample. A dried mixture of potassium and ammonium oxalate (3 gm. ammonium oxalate and 2 gm. potassium oxalate in 100 cc. distilled water) was used as anticoagulant, 0.04 cc. of the solution for each cubic centimeter of blood.

* Eastman Kodak Company.

The following hematologic examinations were made as soon as possible after the removal of the blood:

Red blood cells were counted in the usual manner with Thoma micropipette and Neubauer counting chamber. Hemoglobin was determined by the oxyhemoglobin method in a Klett-Summerson photoelectric colorimeter using 0.025 cc. of whole blood in 5 cc. of distilled water. The reading was made following centrifugalization of the mixture for 30 minutes in order to settle the gross parts of stroma.

Packed red cell values were obtained using the Wintrobe hematocrit tube, centrifugated at 3000 rotations per minute for 30 minutes.

Reticulocyte counts and counts of red blood cells with Heinz corpuscles were made as described below. Brilliant cresyl blue (0.2 gm.) was pulverized in a mortar and dissolved in 100 cc. of 0.6% sodium chloride solution. This gives a final solution of practically the same tonicity as blood plasma, a point of paramount importance in this method. This solution must be filtered twice and kept in very well closed bottle to avoid evaporation and consequent changes in tonicity. One drop of this solution is mixed with 1 drop of blood and observed as a wet preparation between slide and coverslip. Sealing with vaseline is not necessary as the counts must be made within 10 to 15 minutes, during which time the amount of evaporation is negligible. The red blood cells should be allowed to settle to the bottom of the preparation before making the examination. Care must be taken not to prepare thick preparations, since erythrocytes must be isolated to permit careful examination. This can be achieved with practice by using proper amounts of cresyl blue solution and blood when preparing the slide.

The reticulum of reticulocytes appears deeply stained blue against the green color of the protoplasm. Usually, one also observes metachromatic granules stained light red. Heinz corpuscles do not take the stain and appear as highly refractile crystal-like bodies green in color but of greater intensity than that observed in the erythrocyte protoplasm. Heinz granules show very marked Brownian movement when small and appear in any part of the red cell but are usually in the periphery, frequently they protrude from the side of the cell. When large doses of pyrodin are employed to produce anemia, several granules may be seen in the same cell, but with medium or small doses only one granule is seen. These granules are very small after the first injection but grow progressively larger until they attain almost one-fifth of the red blood cell mean corpuscular volume. The granules have a tendency to leave the cell and if the examination is not made as soon as the cells have been sedimented, many granules may be found free in the plasma.

To demonstrate more clearly that the reticulocytes are not attacked by pyrodin a simple blood examination without any staining may be done. Heinz corpuscles appear as described before and the reticulocytes appear as normal untagged erythrocytes, sometimes with a very hyaline granule (metachromatic granule when stained by brilliant cresyl blue) like a white spot within the greenish protoplasm.

Resistance to hypotonic salt solution was determined by mixing 0.025 cc. of blood with 5 cc. of 0.5% solution sodium chloride in distilled water. After standing 3 to 5 minutes the non-hemolyzed red blood cells were centrifugalized and the amount of hemoglobin in the supernatant fluid determined in the Klett-Summerson photoelectric colorimeter. The result was compared with the hemoglobin reading of a tube containing 0.025 cc. of whole blood hemolyzed in 5 cc. of distilled water, hemolysis representing 100%. Because the variation in resistance was very great in these experiments, this method was used in preference to the usual complete hemolysis curves determination.

Estimation of opacity of blood in distilled water was used as a measure of the total volume of Heinz corpuscles formed following pyrodin intoxica-

tion. Since these corpuscles do not dissolve, a turbidity developed when the blood from poisoned animals was diluted in water. After 30 minutes of centrifugalization all the corpuscles had settled to the bottom of the tube, leaving a clear supernatant fluid containing hemoglobin. If the extinction of light before and after centrifugalization is determined in the photoelectric colorimeter (really nephelometry in this case), the difference represents the amount of light absorbed by the Heinz corpuscles, the stroma absorption being negligible.

Experimental Observations. 1. *Formation of Heinz Corpuscles in the Erythrocytes.* In all of 20 breakdown episodes in 13 animals, 20 to 24 hours after pyrodin injections (14 mg. per kilo body weight in rabbits and 19 mg. per kilo in dogs) the presence of Heinz corpuscles was observed in *all* adult red blood cells in the circulation. Furthermore, the young red blood cells (reticulocytes) *never* demonstrated such a structure, even with repeated injections totalling large amounts of the drug (144 mg. per kilo). In one instance (Dog 39-317) when pyrodin (0.9 gm.) was injected at a very low anemia level, the presence of Heinz corpuscles was observed within reticulocytes containing very little amount of reticular substance, *i. e.*, in reticulocytes almost reaching the stage of young adult erythrocytes. However, even in this particular instance, the tagging of a young reticulocyte containing ample reticular substance with a Heinz corpuscle was never observed.

We have seen the formation of Heinz corpuscles *in vitro* with the technique described by Bratley and associates.[6] The appearance of minute corpuscles, 5 or more in each erythrocyte, is easily seen after 10 to 20 minutes. *In vivo*, however, with small and medium doses, a single corpuscle is observed in each red blood cell; this corpuscle increases in size with the severity of intoxication. With massive doses we have observed, as *in vitro*, the formation of 5 or more corpuscles in each red cell. In fixed and stained smears the Heinz corpuscle is invisible when small, but if large enough, it may be seen as a mass staining red (1 to 2 micra in diameter), containing a very minute basophilic dot in the center. This is better shown when the blood is treated with distilled water, staining free corpuscles. Within the red cells the acidophilic structure of the Heinz body fuses with the red color of hemoglobin and then only its central basophilic granule (0.5 to 1.5 micra in diameter) can be seen. In red cell shadows following hemolysis in distilled water, the Heinz body remains within the limits of the cell border. These bodies have a circular shape, they probably are spheres because when a red cell is seen in profile the corpuscle is still circular in shape. As we have pointed out before, the size of the corpuscles increases with the severity of intoxication, therefore the measurement of their volume varies accordingly. We have tried to determine the volume of large Heinz bodies by observing the total volume of packed Heinz bodies from a known amount of hemolyzed blood. Their volume is roughly one-fifth that of the normal red

TABLE 1.—HEMATOLOGIC DATA DURING BREAKDOWN AND REGENERATION IN PYRODIN ANEMIA.

Animal No.:	Rabbit 4.	Rabbit 3.	Dog 39–57 (IV).	Dog 40–248	Dog 37–137 (III).	Dog 37–137 (I).	Dog 39–299 (I).	Dog 39–299 (II).	Dog 39–57 (V).	Dog 39–194	Dog 40–115 (II).	Dog 40–115 (I).	Dog 40–213	Dog 39–57 (III).	Averages in dogs.	
															Below 40 mg. pyrodin per kilo.	Above 40 mg. pyrodin per kilo.
Blood picture before breakdown: R.B.C. (mill. per c.mm.)	5.7	6.3	6.2	6.2	5.8	5.5	7.3	6.9	6.2	5.4	5.6	5.2	5.8	5.4	6.0	5.9
Hemoglobin (gm.)	11.2	13.4	13.8	13.6	13.6	13.5	9.9	12.9	13.4	12.0	12.7	11.6	12.9	12.2	13.6	12.3
Packed red cells (%)	41.5	44	47	40.5	43.5	40.5	38.5	42	50	42	44	42	42	42.5	43.5	42
Number pyrodin injections, amount used in each (mg.)	5–45	6–45	1–400	2–300	2–200	2–200 1–300	4–300	4–300	5–300	6–300	6–300	8–300	6–300	7–400		
Amount pyrodin per kilo body weight (mg.)	80	84	27	38	39	42	67	67	100	105	106	141	144	187	35	107
Days necessary to complete disappearance of red cells with Heinz corpuscles from the circulation	8	9	18	12	15	9	13	12	14	13	12	11	12	14	15	12
Average daily destruction (mill. per c.mm.)	0.713	0.700	0.344	0.517	0.387	0.611	0.562	0.575	0.443	0.415	0.467	0.474	0.483	0.386	0.416	0.490
Lowest level of anemia: R.B.C. (mill. per c.mm.)	1.1	1.0	3.2	3.4	3.2	1.5	1.6	1.3	1.5	1.7		1.5	1.4	1.7	3.3	1.4
Hemoglobin (gm.)	2.8	2.4	7.5	7.4	7.4	3.7	3.4	2.7	3.5	3.1		3.0	2.4	3.8	7.4	3.2
Packed red cells (%)	16	14	27	26.5	26	14	14.5	12	15	19		19	15.5	17.5	26.5	16
When observed (days after first injection)	6	6	10	8	8	6	7	8	10	7		8	8	10	8	8
Beginning of regeneration (days after 1st injection)	2	2		3	4	3	3	4	5	3	4	4	3	4	3–4	3–4
End of first regeneration period: R.B.C. (mill. per c.mm.)	3.9	4.5		5.0	4.9	4.7	5.1	4.1	4.4	4.2		4.6	4.3	4.6	5.0	4.5
Hemoglobin (gm.)	9.6	10.4		11.2	12.3	11.8	10.9	9.8	9.6	9.7		9.1	9.5	9.4	12.0	10.0
Packed red cells (%)	38	40		38	40	41	39	35.5	39	39		38	39.5	38	39	38.5
When observed (days after beginning of regeneration)	15	15		10	11	15	16	13	16	21		15	15	16	10–11	16
Average daily regeneration (mill. per c.mm.)	0.260	0.300		0.500	0.445	0.314	0.319	0.316	0.275	0.200		0.317	0.286	0.287	0.472	0.280

cell. After hemolysis and centrifugalization, the Heinz corpuscle appears as a lightly colored mass, strongly bound together in the bottom of the tube. After long centrifugalization it is very difficult to get them again in suspension, so firmly are they coherent.

2. *Measure of Destruction and New Formation of Red Blood Cells.* Findings observed during destruction and regeneration of red blood cells are summarized in Table 1. Varying total amounts of pyrodin were employed in dogs for each breakdown episode ranging from 27 to 187 mg. per kilo body weight. As concerns the results, the

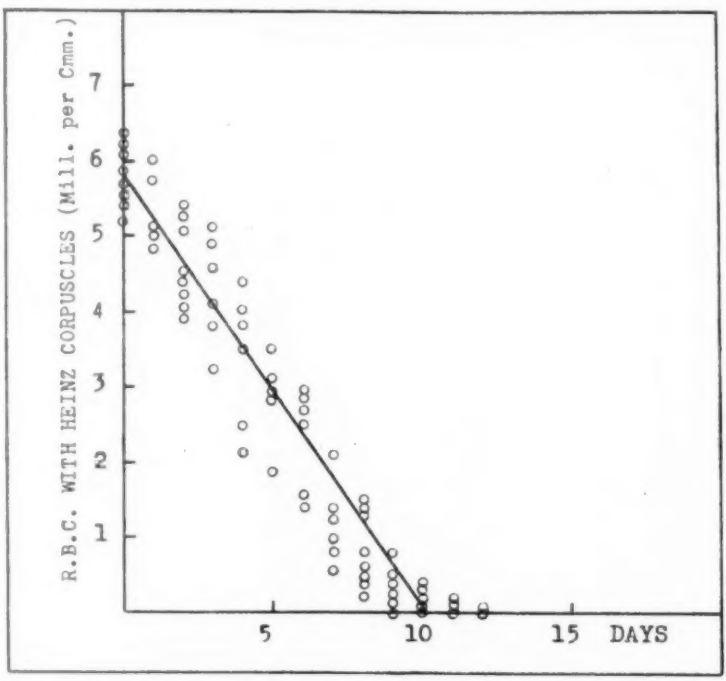

CHART 1.—The disappearance from the blood stream of erythrocytes containing Heinz corpuscles.

doses can be regarded as: small (27 to 40 mg.) and large (above 40 mg.). In small doses the rate of daily average disappearance is 0.416 million R.B.C. per c.mm., taking 12 to 18 days for complete disappearance. In large doses the rate of daily average disappearance is 0.490 million R.B.C. per c.mm., taking 9 to 14 days for complete disappearance.

The lowest anemia level in the first group (small doses) was twice as low as that in the second group (large doses). In both groups, it was attained 7 to 10 days after the first injection of pyrodin.

Regeneration starts 3 to 4 days after the beginning of intoxication

and is more rapid with small doses (daily output of 0.472 R.B.C. million per c.mm.) than with large (daily output of 0.290 R.B.C. million per c.mm.). The first period of regeneration is characterized by a steady increase in the number of the red blood cells. In the following period the rate of production is slowed down and continues until a normal level is reached. The end of the first period is attained in dogs in 10 to 11 days with small doses, in 16 days with large.

In rabbits, the disappearance of the erythrocytes containing Heinz bodies is much more rapid (8 to 9 days), the lowest anemia level is even lower than that in dogs, and is observed earlier (6 days after

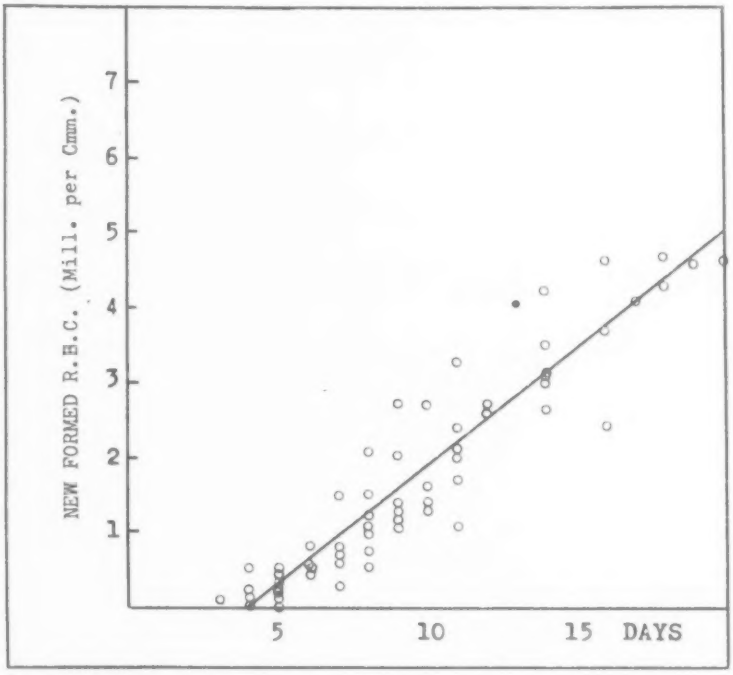

CHART 2.—The appearance of new formed red cells following pyrodin anemia.

the first injection). Regeneration begins sooner (2 days after the first injection) but is almost at the same rate as that observed in the second group of dogs.

Chart 1 presents the disappearance of the red blood cells with the Heinz bodies from the blood stream of 8 dogs. Numerical decrease of these cells in the circulation is proportional to the time elapsed, as shown by the straight line. Chart 2 illustrates the appearance of newly formed cells in the blood stream of 8 dogs. It shows that regeneration (to subnormal levels values of 4 to 5 million R.B.C. per c.mm.) is proportional to time. To illustrate the picture during

the destructive phase and the subsequent regenerative phase, complete figures in 1 dog (No. 40–213) are presented in Table 2.

TABLE 2.—DETAILED HEMATOLOGIC OBSERVATIONS DURING DESTRUCTIVE AND REGENERATIVE PHASES OF PYRODIN ANEMIA IN A TYPICAL EXPERIMENT. (DOG 40–213.)

Days of observation.	R.B.C. (mill. per c.mm.).	Hb. (gm. per 100 cc. blood).	Packed red cells (%).	Mean corp. volume (cubic micra).	Mean corp. hemoglobin (micromicrograms).	Mean corp. hemoglobin concentration (%).	Reticulocytes (mill. per c.mm.).	New formed adult R.B.C. (mill. per c.mm.).	R.B.C. with Heinz corpuscles (mill. per c.mm.).	Hemolysis in 0.5% solution NaCl (%).	Opacity of Heinz corpuscles (extinction).
0*	5.8	12.9	42	73	22.4	31	0.10	..	..	10	0
1*	4.8	9.6	36.5	74	20.0	26	0.14	..	4.66	31	16
2*	4.6	9.5	35	76	20.6	27	0.18	..	4.42	41	49
3*											
4*	3.0	5.6	26	87	18.7	22	0.54	..	2.46	72	71
5*	2.4	4.5	23	96	18.7	20	0.41	..	1.99	78	66
6	2.0	3.3	19.5	97	16.5	17	0.52	..	1.48	73	57
7	1.6	2.9	17.5	110	18.5	17	0.80	..	0.80	74	45
8	1.4	2.3	15.5	111	16.8	15	1.08	0.07	0.25	54	25
9	1.5	2.7	17	113	18.0	16	1.02	0.32	0.10	36	17
10	1.7	3.3	18.5	109	19.4	18	0.88	0.75	0.07	22	12
11	2.0	4.1	21.5	107	20.5	19	0.64	1.34	0.02	5	8
12	2.7	5.8	29	107	21.5	20	0.81	1.89	0	9	0
14	3.1	7.3	34	109	23.4	21.5	0.59	2.51	0	10	
16	3.7	8.3	36	97	22.4	23	0.30	3.40	0	22	
18	4.3	9.5	39.5	92	22.1	24	0.09	4.21	0	13	
24	5.1	11.5	43	84	22.6	27	0.02	5.08	0	8	
29	4.9	10.9	40	82	22.2	27	0.02	4.88	0	15	
33	5.0	10.7	40.5	81	21.5	26.5	0.02	4.98	0	5	
37	5.8	13.2	45.5	78	22.7	29	0.03	5.77	0	15	

* Acetylphenylhydrazine injections (300 mg.).

3. *Measure of Resistance of Poisoned Red Cells Toward a Hypotonic Salt Solution.* In Chart 3 the percentage of hemolysis observed in hypotonic (0.5%) saline solution during 14 breakdown episodes in 10 dogs is plotted against time. The results indicate that 11 days after the first injection, the behavior of circulating erythrocytes toward this hypotonic solution has again become normal. The fragility of the cells containing Heinz bodies increases with successive pyrodin injections, in some dogs reaching 95% hemolysis on the fifth day. As the new formed cells start to enter the circulation on the third or fourth days after the beginning of the injections, a mingling with the damaged cells occurs, resulting in a progressive return of fragility towards normal from the sixth day on. Since all poisoned red cells (very fragile to hypotonic solution) have completely disappeared from circulation on the eleventh day, the fragility test is again normal at this time.

4. *Quantitative Measure of the Amount of Heinz Corpuscles in Circulation Indicative of Severity of Intoxication.* Chart 4 shows the

degree of red blood cells intoxication during the breakdown period, as measured by the total volume of Heinz corpuscles in circulation. It represents the results of 15 breakdown episodes in 10 dogs. As in Chart 3, illustrating fragility, the result shows a maximum intoxication on the fifth day after the first injection and complete disappearance of the corpuscles on the eleventh day. The first half of the curve shows a progressive increase in the volume of corpuscles as indicated by the increase in the opacity of the fluid when blood was mixed with distilled water. From the fifth day on opacity progressively decreased coinciding with the disappearance of the red blood cells with the Heinz bodies from the blood stream.

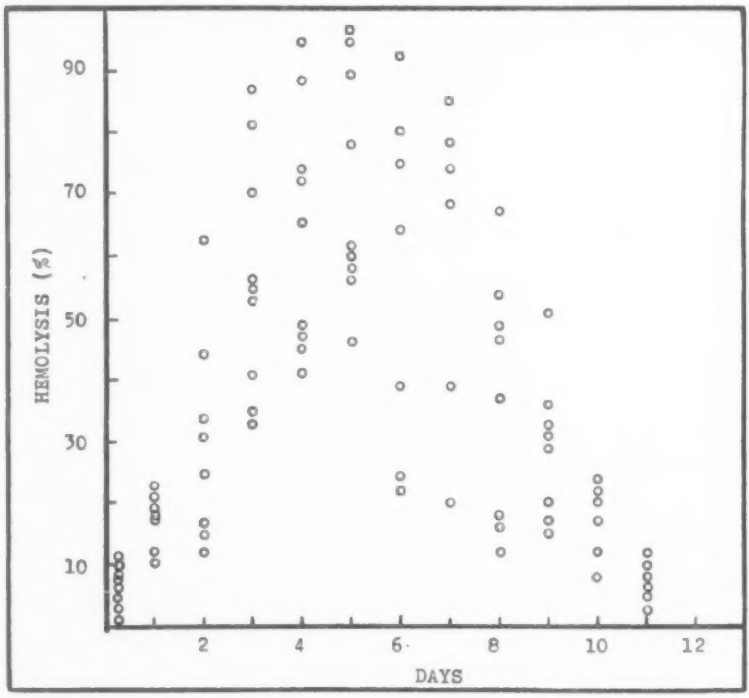

CHART 3.—Behavior of poisoned erythrocytes toward 0.5% saline solution.

5. *Higher Peaks of Reticulocytosis Observed During Regeneration.* Table 3 shows the peak concentration of reticulocytes in the regenerative periods. The number of reticulocytes in the peripheral blood seems to be proportional to the total amount of pyrodin given, or perhaps to the time in which this total amount has been administered.

Dog 40–115 could not be followed after the figures presented in Table 3 were reached. We believe, however, that the figure shown

does not represent the maximum reticulocytosis obtainable in this case.

TABLE 3.—HIGHEST RETICULOCYTOSIS OBSERVED AFTER PYRODIN ADMINISTRATION.

Dog No.	Peak of reticulocytosis.		Lowest anemia level, mill. per c.mm.	Number of pyrodin injections.	Amount of pyrodin per kilo of body weight (mg.).
	%	Mill. per c.mm.			
37–137 (II)	46	0.490	3.2	2	39
40–248	12	0.600	3.4	2	38
37–137 (I)	26	0.730	1.5	3	42
39–299 (I)	32	0.730	1.6	4	67
39–299 (II)	35	0.840	1.3	4	67
40–213	77	1.000	1.4	6	144
39–194	79	1.400	1.7	6	105
40–115 (I)	75	1.100	1.5	8	141
Rabbit No.					
4	88	0.970	1.1	5	80
3	87	1.300	1.0	6	84

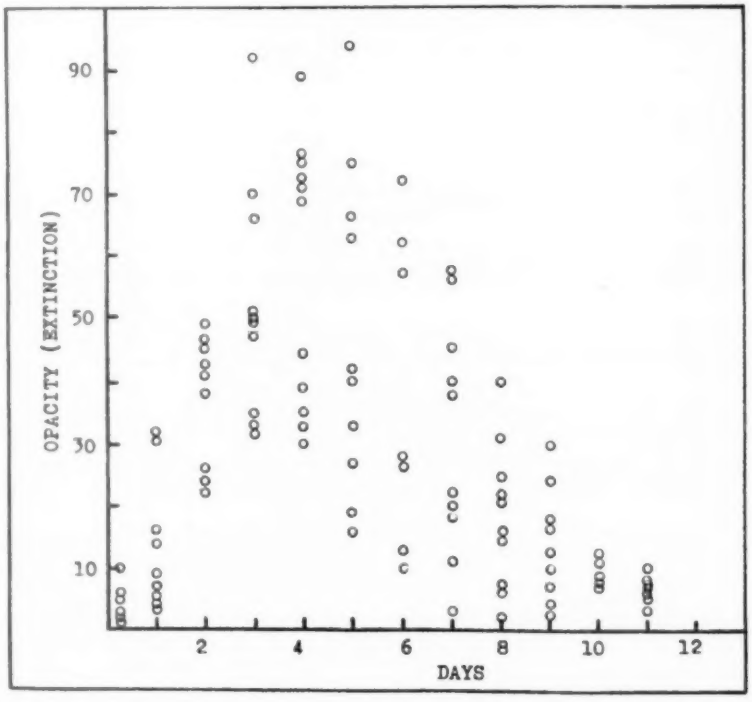

CHART 4.—Measure of turbidity developed by poisoned blood when treated with distilled water. Opacity was measured by light extinction according to the extinction scale of the Klett Summerson photoelectric colorimeter.

6. *Measure of Reticulocyte Mean Corpuscular Volume in Dogs With Normal Iron Reserves and in Dogs With Depleted Iron Stores.* Chart 5 shows changes in volume of reticulocytes in their development to

the adult erythrocyte. The results presented are an average of the observations made on 7 non-anemic dogs (Nos. 40–213, 37–137, 40–115, 39–194, 40–248, 39–319 and 39–57) and on 2 anemic dogs with depleted iron reserves (Nos. 39–299 and 39–26).

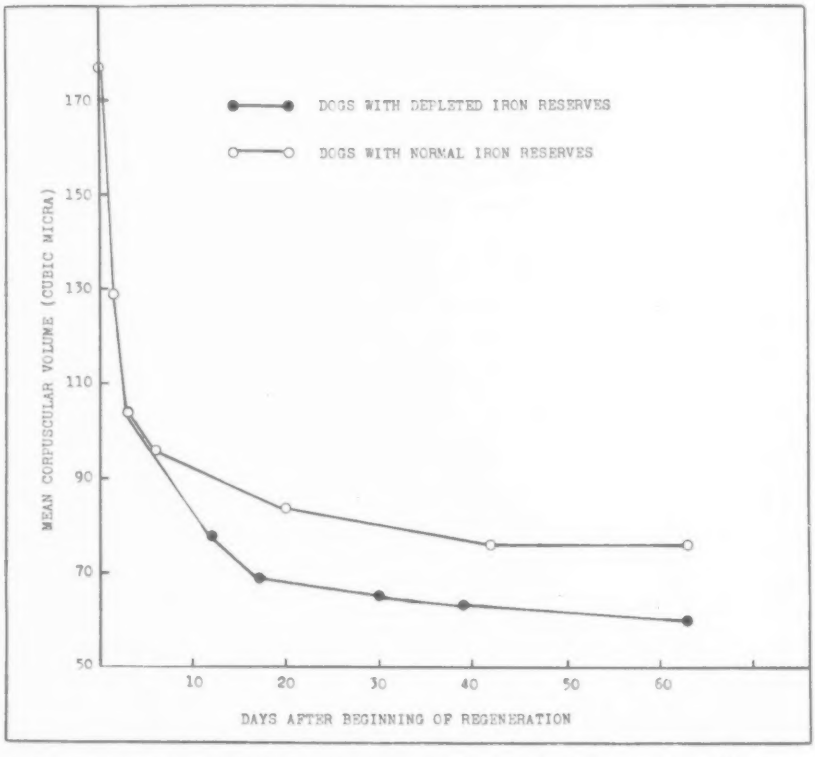

CHART 5.—Mean corpuscular volume changes during blood regeneration following pyrodin anemia.

Having determined the following data: (*a*) mean corpuscular volume of red blood cells with Heinz corpuscles; (*b*) the mean corpuscular volume of the mixture of reticulocytes and red blood cells with Heinz corpuscles; and (*c*) the ratio between these two groups of cells, we can then calculate the volume of reticulocytes by the following formula:

$$V = \frac{V_1.100 - (V_2.P)}{P_1}$$

where:

V = Mean corpuscular volume of reticulocytes.
V_1 = Mean corpuscular volume of all red blood cells.
V_2 = Mean corpuscular volume of red blood cells with Heinz corpuscles.
P = Percentage of red blood cells with Heinz corpuscles.
P_1 = Percentage of reticulocytes.

In Chart 5 we can see the rapid change in the mean volume of reticulocytes in the first 3 days of circulation, during which time it decreases from 176 cubic micra to 104 cubic micra. From this point on a difference was observed between anemic and non-anemic dogs. In non-anemic dogs with normal iron reserves the volume of these new formed cells (at this point non-reticulated young erythrocytes) decreases slowly until it reaches normal levels (74 to 78 cubic micra). This occurs between 30 to 40 days after the beginning of regeneration, and does not change appreciably from this level. In anemic dogs, however, with iron reserves depleted by bleeding and kept at low hemoglobin levels for a long period of time before the pyrodin was given, the mean corpuscular volume reached a normal level much earlier (10 to 20 days) and continues to decrease until a microcytic level was reached (58 to 63 cubic micra) identical to that level observed before the drug was given.

7. *Observations on the Maturation of Reticulocytes in the Blood Stream.* During the period in which daily injections of pyrodin were given, all red blood cells appeared as reticulocytes or as erythrocytes containing Heinz corpuscles. Two to 3 days after the last injection another type of erythrocyte was observed in the circulation: a large cell of about the same size as the reticulocyte, undamaged (homogeneous plasma without any granule or vacuole). Some of these cells contained a very small amount of reticular substance while others appeared as complete adult erythrocytes, with no reticulum whatever. This new kind of cell seems to result from the reticulocyte evolution, as indicated by the presence of all types of intermediate stages.

For differentiation purposes we called these cells "new formed adult erythrocytes." At this phase, then, three different kinds of red blood cells were observed: reticulocytes, new formed adult erythrocytes and damaged erythrocytes (red blood cells containing Heinz corpuscles). Table 4 represents quantitative determinations of these three kinds of red blood cells during 7 breakdown episodes in 5 dogs.

8. *Pathologic Examinations.* Sections of some organs have been made in dogs in which death occurred during the red blood cell breakdown period. The anemia was very pronounced in these dogs, 1 day before their deaths; red blood cells ranging from 1.5 to 2 million per c.mm.; hemoglobin from 3 to 3.5 gm. per 100 cc. of blood. In the non-splenectomized dogs (Nos. 40–213, 39–317 and 39–1) we have found large amounts of iron pigment, as well as some intact red blood cells, within the reticulo-endothelial cells of the spleen, liver and bone marrow. The greatest amounts were found in the spleen. In the sections treated with potassium ferricyanide, deposits of intense blue color could be found with intact red blood cells within the same phagocytic cell. This picture was not found very frequently but could be seen without much difficulty. In 1 splen-

TABLE 4.—DETERMINATIONS OF NEW FORMED AND DAMAGED ERYTHROCYTES IN PYRODIN ANEMIA.

Days of observation.	Dog 37–137 (I).				Dog 37–137 (II).				Dog 39–299 (I).				Dog 39–299 (II).				Dog 39–194.				Dog 40–248.				Dog 40–213.			
	(1)	(2)	(3)	(4)	(1)	(2)	(3)	(4)	(1)	(2)	(3)	(4)	(1)	(2)	(3)	(4)	(1)	(2)	(3)	(4)	(1)	(2)	(3)	(4)	(1)	(2)	(3)	(4)
0	5.5*	0.04	0	..	5.8*	0.01	0	..	7.3*	0.01	0	..	6.9*	0.01	0	..	5.4*	0.10	0	..	6.2*	0.01	0	..	5.8*	0.10	0	
1	4.9*	0.05	0	4.90	5.0*	0.01	0	5.00	6.5*	0.01	0	6.50	5.4*	0.01	0	5.39	*	..	0	..	5.7*	0.01	0	5.7	4.8*	0.12	0	4.68
2	4.0*	0.08	0	3.82	4.3	0.02	0	4.28	*	..	0	..	*	..	0	..	4.0*	0.10	0	3.93	5.3	0.01	0	5.3	4.6*	0.18	0	4.42
3	3.0	0.09	0	2.91	4.6	0.05	0	4.55	6.0*	0.12	0	5.88	3.9*	0.01	0	3.89	3.6*	0.18	0	3.42	..	..	..	..	.. *	..	0	
4	2.0	0.26	0	1.74	4.0	0.08	0.12	3.80	..	..	..	..	..	..	..	..	2.8*	0.67	0	2.13	3.9	0.24	0.08	3.58	3.0*	0.54	0	2.46
5	1.6	0.40	0.13	1.07	..	..	..	..	3.1	0.30	0	2.80	2.3	0.18	0.04	2.08	*	..	..	..	..	..	..	..	2.4*	0.41	0	1.99
6	1.5	0.33	0.50	0.67	3.6	0.14	0.61	2.81	..	..	..	..	..	..	..	..	2.2	0.73	0	1.47	3.5	0.39	0.46	2.65	2.0	0.52	0	1.48
7	1.7	0.39	1.09	0.22	3.4	0.20	1.10	2.10	1.6	0.33	0.29	0.98	..	..	..	..	1.7	1.17	0	0.53	..	..	..	..	1.6	0.80	0	0.80
8	2.1	0.55	1.55	0.02	3.2	0.16	1.60	1.44	2.1	0.57	0.98	0.55	1.3	0.45	0.57	0.28	1.8	1.42	..	..	3.4	0.27	1.90	1.23	1.4	1.08	0.07	0.25
9	2.8	0.73	2.04	0	3.2	0.19	2.20	0.81	2.3	0.73	1.33	0.24	1.4	0.45	0.93	0.05	1.7	1.19	0.34	0.17	..	..	..	..	1.5	1.00	0.32	0.18
10	..	..	..	..	..	..	..	..	2.8	0.64	2.07	0.09	..	..	..	..	1.7	0.63	0.99	0.08	3.5	0.24	2.90	0.36	1.7	0.89	0.75	0.06
11	3.3	0.63	2.67	0	3.8	0.38	3.15	0.27	..	..	..	..	2.4	0.84	1.56	0.01	1.7	0.60	1.04	0.06	..	..	..	..	2.0	0.63	1.36	0.01
12	..	..	..	..	..	..	..	..	3.4	0.41	2.94	0.05	..	..	..	..	..	..	..	..	..	..	..	..	2.7	0.81	1.89	0
13	..	..	..	..	3.9	0.31	3.60	0.01	..	..	..	..	..	..	..	..	..	..	..	0	5.0	0.60	4.40	0	..	..	..	..
14	3.7	0.14	3.33	0	..	..	..	..	4.2	0.42	3.78	0	3.3	0.30	3.00	0	2.4	0.58	1.82	0	..	..	..	..	3.1	0.59	2.51	0
15	..	..	..	..	4.9	0.49	4.41	0	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
16	..	..	..	..	..	..	..	..	4.6	0.18	4.42	0	..	..	..	..	..	..	..	..	..	..	..	..	3.7	0.30	3.40	0
17	..	..	..	..	..	..	..	..	..	..	..	..	4.1	0.16	3.92	0	3.0	0.27	2.73	0	..	..	..	..	..	..	..	..

Column (1) = Total R.B.C., mill. per c.mm. Column 2 = reticulocytes, mill. per c.mm. Column 3 = New formed adult R.B.C., mill. per c.mm. Column (4) = R.B.C. with Heinz corpuscles, mill. per c.mm.

* Acetylphenylhydrazine injections.

ectomized dog we observed (No. 39–152) a considerable increase in the size of Kupffer cells in the liver and reticulo-endothelial cells of the bone marrow. All of them contained large amounts of iron pigments and some phagocyted erythrocytes.

Discussion. *Blood Destruction.* In studying the mechanism of the action of hydrazine derivatives upon the blood, it is of paramount importance not to compare results obtained with different drugs, or different amounts of substances, or, finally, with different methods of administration. For instance, Itoh,[21] with 10 to 50 mg. of phenylhydrazine hydrochloride given intravenously in dogs, obtained a drop from 7 million red blood cells per c.mm. to less than 1 million in 24 hours, with massive hemolysis in the serum. Alcobe,[1] giving 10 mg. of phenylhydrazine hydrochloride per kilo body weight (no information about the method of administration), obtained massive hemolysis. Formation of methemoglobin is described by Dreschfeld[12] and some other authors. We found a similar picture in 1 dog (No. 39–262) into which we injected subcutaneously a massive dose of 2 gm. of pyrodin in one single injection. This dog died 5 days after the injection.

When one employs, however, non-lethal doses of a hydrazine derivative, quite a different picture is obtained. Massive hemolysis in the blood stream with the presence of free hemoglobin in the plasma is not demonstrable but one may see very slight traces during the beginning of the intoxication. We observed a marked tendency to spontaneous hemolysis *in vitro* with intoxicated red cells. To demonstrate hemoglobinemia properly it is important that the blood be centrifuged as soon as possible following removal from the body. Even in these cases, at times one may observe a small layer of hemolysis in the upper part of the packed red cells in the hematocrit tube. In some cases 10 to 15 minutes at room temperature is sufficient to produce some degree of hemolysis.

A characteristic finding of that type of intoxication pointed out by Heinz[20a,b] is the presence of a refractile granule in the red blood cells. This author observed the same phenomenon when using other hydrazine derivatives. Certain properties of these Heinz bodies have been described by Hartwick[18] and a detailed chemical analysis has been made by Kunkle.[22] We have confirmed the presence in these of very minute amounts of iron. Furthermore, we observed the non-formation of these bodies in the reticulocytes; a rough determination of its volume during the most acute phase of intoxication as well as the determination of the total amount of Heinz bodies (nephelometric method) during different phases of the poisoning.

In the most important papers concerning treatment of polycythemia vera with hydrazine derivatives, we were unable to find any reference to the presence of Heinz corpuscles.[2,3,14,15a,b,c,16,24,25,29–31] It seems important to us to know whether all the erythrocytes are

corpuscles, mill. per c.mm.
* Acetylphenylhydrazine injection

damaged by the drug in the treatment of polycythemia vera, as occurs in the experimental anemias related in this paper.

In our experiments the destruction of erythrocytes containing Heinz corpuscles was complete within 9 to 18 days after the first injection. This interval appears to be proportional to the amount of pyrodin administered. It is worth mentioning that the constancy of the rate of destruction is always proportional to the time elapsed after the beginning of intoxication. We were unable to find in the literature figures relating to the destruction of erythrocytes containing Heinz corpuscles. Duesburg,[13] injecting 77 mg. of pyrodin per kilo into rabbits, obtained a drop in the red blood cell count similar to those observed by us.

There are two different ways to observe the progress of intoxication and consequent disappearance of the erythrocytes: (*a*) determination of the total amount of Heinz corpuscles; (*b*) fragility towards hypotonic salt solution. The results show a correlation between the size of the Heinz corpuscles and the fragility toward 0.5% salt solution. Fragility decreases as the total amount of Heinz bodies increases. Following the beginning of regeneration, a progressive number of new red cells are launched into the circulation, these show normal behavior toward hypotonic salt solution and do not contain Heinz corpuscles. During this stage, the blood is a mixture of normal and greatly damaged cells. As the damaged cells disappear, the total volume of Heinz corpuscles tends to diminish and the fragility again becomes normal. This change toward normalcy of the blood occurs between 9 and 18 days following the first injection, as is indicated by the close relationship between three types of observation: 1, morphologic demonstration of Heinz bodies by means of direct microscopic examination; 2, determination of the amount of Heinz corpuscles by nephelometric method; 3, behavior toward hypotonic salt solution.

The lack of any appreciable amount of free hemoglobin in the plasma and the progressive disappearance from the circulation of erythrocytes containing Heinz bodies, seems to indicate a slow, continuous removal of these damaged cells as *whole* cells from the blood stream. Long[23] and Alcobe[1] demonstrated erythrophagocytosis in this type of anemia. We have confirmed these observations and were impressed with the intensity of this phenomenon. In order to demonstrate that the enormous amount of iron pigment found in the reticulo-endothelial cells is the result of phagocytosis of the red blood cell and not of free hemoglobin, we sacrificed a rabbit 24 hours after the first injection of pyrodin. We expected to find a greater number of intact erythrocytes in the reticulo-endothelial cells. The expected picture was not seen, probably because the hemoglobin of the phagocytized erythrocytes was rapidly converted into bilirubin with consequent deposition of iron as shown in experiment done in dogs with gall-bladder renal fistula.[10] In these dogs there

was a marked increase of bilirubin output in the urine eliminated during a 24-hour period following the first injection. Our interpretation of phagocytosis as the main mechanism of destruction in this type of anemia is based upon the frequent finding of red cells within reticulo-endothelial cells containing iron pigment as well.

Blood Regeneration. The reticulocytes increase in number between the third and fourth days following the first injection. The number of new cells formed each day appears to be quite constant, at least for the 10 first days of the regenerative period. As we[8] and Bodansky[4] have observed during treatment of hookworm anemia and other hypochromic anemias, the reticulocytes have a greater volume than the adult erythrocytes. Bodansky and Dressler[5] found an increase of the water content in the reticulocytes as compared with adult erythrocytes. An approximate idea of the mean corpuscular volume of the reticulocytes as presented in this paper, indicates a volume twice that of the adult erythrocyte. The evolution of the reticulocyte toward the adult erythrocyte seems to be accompanied by a rapid decrease in mean corpuscular volume. A modification of its shape must also occur if we take into consideration the result obtained in a recent study[9] of the fragility in hypotonic solutions of new formed cells tagged with radioactive iron. This interpretation is based upon the widely accepted explanation of the mechanism of hemolysis toward hypotonic solutions, emphasizing the relationship between the shape of red cells and their behavior in hypotonic solutions.[7,17,26]

The amount of hemoglobin in the reticulocytes seems to be the same as that in adult erythrocytes, thus indicating a lower hemoglobin concentration for the reticulocyte as compared with the mature erythrocyte.

In animals rendered anemic by bleeding, erythrocytes are microcytic and poor in hemoglobin. In these animals the regeneration following hydrazine shows the presence of reticulocytes of the same size and hemoglobin content as observed in reticulocytes of a normal dog. This finding indicates a normal capacity to form red cells in such conditions. If this takes place during the physiologic formation of new red cells in this type of anemia (hypochromic, microcytic) it might indicate that the erythrocytes become smaller and hypochromic in the blood stream through loss of hemoglobin while in the circulation. It is surprising to note how great the difference in the distribution of hemoglobin is before and after administration of hydrazine. For instance, in anemic Dog 39–299, 7.4 gm. of hemoglobin per 100 cc. of blood was distributed among 6.5 millions R.B.C. per c.mm. In contrast, during the regenerative phase following the administration of pyrodin, we found the same amount of hemoglobin (7.4 gm.) distributed among only 3.4 millions R.B.C. per c.mm., *i. e.*, half the number of erythrocytes found previously.

It is a well-known fact that the greatest experimental reticulo-

cytosis is obtained with anemias produced by hydrazine derivatives. Does this mean a more violent regeneration than other types of experimental hemolytic anemias, as produced for example by intravenous injections of distilled water? We believe that the percentage of reticulocytes is much higher than in other types of anemia, because with hydrazine anemia we have a progressive concentration of reticulocytes in the blood due to the fact that destruction occurs only in non-reticulated cells. The resistance of the reticulocytes to the drug makes these cells immune to damage, while on the other hand the adult red cells are rapidly disappearing from the blood stream.

However, it is not only a high percentage of reticulocytes that one observes in this anemia, but also a truly great increase in their total number. In Table 3 it is shown that the highest absolute numbers are obtained with a large number of injections, while lower values are obtained only when a few injections have been given. Table 4 indicates that when 6 injections have been administered (Dogs 31–194 and 40–213) there resulted 1.42 and 1.08 million reticulocytes per c.mm. before any evolution toward adult erythrocyte was observed. This seems to indicate either a paralyzing property of pyrodin in the maturation phase reticulocyte—adult erythrocyte, occurring in the blood stream, or a hindering property on the bone marrow capacity to form complete adult red cells. As is indicated in Table 4, maturation of reticulocytes always occurred between 48 and 72 hours after the last injection. This constancy could perhaps be interpreted as one more indication that the span of the reticulocyte stage is of that order of magnitude. If we consider that the reticulocytes are still subjected to the drug for a certain period of time following the last injection, it is quite possible that the maturation time in physiologic conditions might be around 24 hours, as observed in humans by some authors.[11,19,27,28]

We believe that this type of anemia would give greater information concerning the duration of the reticulocyte stage in the blood stream if careful morphologic observations were made at short intervals (12 hours) during the period of regeneration. In this way quantitative data could be obtained regarding the transformation of the reticulocyte into a newly formed adult erythrocyte. Also the question as to whether the red cell always leaves the bone marrow as a reticulocyte or also sometimes as a complete adult erythrocyte under normal conditions could perhaps be elucidated by this experimental anemia.

Summary. The administration of pyrodin produces no marked differences in the destruction and regeneration of erythrocytes between normal, splenectomized, renal fistula dogs and those with severe hypochromic anemia.

Destruction Phase. 1. In dogs or rabbits all red blood cells other than reticulocytes are damaged 24 hours after injection of pyrodin, as shown by the presence of Heinz bodies in the cells.

2. Damaged cells became progressively more fragile in 0.5% NaCl solution in distilled water.

3. In a given anemia the Heinz bodies increase in size with the severity of intoxication that follows repeated doses; the Heinz body is larger in the severely intoxicated animal than in one less severely intoxicated. Heinz bodies produce increased turbidity when blood is laked in distilled water.

4. Damaged cells disappear progressively and completely from the blood stream in 12 days, on the average, after beginning the poisoning. Evidence for this is obtained by direct morphologic examination; by return to normal behavior toward hypotonic salt solution, as well as by disappearance of the turbidity of distilled water due to insolubility of Heinz bodies.

5. Doses of pyrodin employed did not cause appreciable hemolysis (as seen by the appearance of the plasma) throughout the intoxication process.

6. Pathologic examinations of animals sacrificed or dead at different stages of the intoxication showed large amounts of iron pigment and some erythrophagocytosis in the spleen, liver and bone marrow.

7. The mechanism of destruction in this type of anemia apparently acts primarily by damaging erythrocytes. These cells become like foreign bodies and are phagocytosed by cells of the reticuloendothelial system.

Regeneration Phase. 1. The regeneration begins 3 to 4 days following intoxication and is maintained for 10 to 15 days at a high rate, putting into circulation on the average 0.300 million R.B.C. per c.mm. daily.

2. Mean corpuscular volume of reticulocytes could be roughly estimated as double that of adult erythrocytes.

3. It has been shown that dogs with severe microcytic, hypochromic anemia could produce reticulocytes with normal mean corpuscular volume, containing normal amount of hemoglobin, during regeneration after pyrodin anemia. However, microcytosis could be again observed after some time.

4. If all erythrocytes leaving the bone marrow are reticulocytes, then pyrodin seems to have a paralyzing effect on the maturation of reticulocytes to normal adult erythrocytes. This fact, together with the specific destruction of adult red blood cells, seems to explain the high percentage of reticulocytes in this type of anemia.

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RESULTS OF CHEMOTHERAPY IN SUBACUTE BACTERIAL ENDOCARDITIS.

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Lichtman and Bierman[6] have recently assembled the reported largest series of experiences in the treatment of subacute bacterial endocarditis with sulfonamide drugs. Among 198 cases receiving simple chemotherapy there were 12 (6%) recoveries. Among 89 other cases in which such chemotherapy was combined with other measures—intravenous heparin, physically induced hyperthermia, intravenous typhoid vaccine or radiotherapy—there were 14 (16%) recoveries.

It is our purpose to add to these reports the experiences in the treatment of 36 cases of subacute bacterial endocarditis in this hospital who received one of the sulfonamide drugs for more than 1 week, more than 2 weeks before death. Among these were 1 probably proven case with recovery and 1 death with cerebral hemorrhage in a patient who was receiving continuous heparin administration.

Several patients received more than one sulfonamide drug; 23 received sulfapyridine; 13 received sulfanilamide; 7 received sulfathiazole; 4 received sulfamethylthiazole; 1 received neoprontosil and 1 received sodium paranitrobenzoate.

As has been a common experience, particularly during the administration of sulfapyridine, there was frequently a subsidence of fever without apparent effect on the course of the disease. With the exceptions to be noted, the temperature never remained completely normal for more than a very few days. The antipyretic effect of sulfapyridine has been noted by other observers.[1]

When colony counts were done, there was commonly a decrease in the number of organisms demonstrated in the blood stream. Five patients had one or more negative blood cultures during chemotherapy while they continued to be febrile. These were probably falsely negative cultures. Janeway,[4] in reporting a method to avoid such falsely negative blood cultures, remarked on the inhibiting effect of previous exposure of organisms to sulfanilamide and of the sulfanilamide present in the blood sample upon the growth of a small number of organisms.

Three patients had complete remissions of fever with repeatedly negative blood cultures for 18 days, 19 days, and 2 months, respectively. One of these was the case with cerebral hemorrhage, to be described. The other 2 relapsed and died in spite of continued chemotherapy.

Case Reports. CASE 1.—W. P., 23-year-old male student, was admitted to the hospital on October 15, 1939. Two years previously he had received here the diagnoses of congenital heart disease, tetralogy of Fallot, moderate cardiac enlargement, right bundle branch block and fair exercise capacity.

For 1 week he had noted gradually increasing ease of fatigue and aching of the back and legs. Four days before admission he had two episodes of sharp pain in the right paraumbilical region, followed by a mild residual pain. On the morning of admission he experienced a sudden, severe pain in the lower anterior chest, on both sides, which was greatly aggravated by inspiration. During that day he had eight shaking chills.

Physical Examination. The patient appeared to be acutely ill, in distress, and was deeply cyanotic. Respirations were shallow and rapid. No abnormal pulmonary signs were detected. The apex impulse was in the fifth interspace, 8 cm. to the left of the mid-sternum. There was a pronounced systolic thrill over the precordium, most prominent over the upper left portion of it. A loud harsh murmur with similar location of maximum intensity almost completely obliterated the first heart sound. It was transmitted well over the anterior chest and but slightly to the neck vessels. There was mild tenderness in both costovertebral angles and over the right side of the abdomen. There was marked clubbing of the fingers and toes.

Laboratory Data. Roentgen rays of the chest on the 2d, 10th, and 20th hospital days showed no evidence of a pulmonary lesion. Eleven of 16 urinalyses showed a trace or more of albumin. On the 22d and 23d hospital days there were 10 to 15 red blood cells per high power field in the centrifuged urinary sediment. The white blood cell count was 10,500 and remained at that level until the third week of hospitalization, when it ranged between 12,000 and 13,500. Hemoglobin was 150% (Sahli). The red blood cell count was 8,770,000. Four blood cultures in the first 3 weeks showed no growth. *Strep. viridans* grew in the blood cultures taken on the 20th and 21st hospital days. Subsequent blood cultures showed no growth.

Course. During the first 36 hours the temperature varied between 102° and 104.5° (rectal). Then there was an abrupt drop in the temperature

following which there was a daily maximum of 99° to 99.5° (oral) until the 20th hospital day. The patient then had a severe chill with a fever of 105° (rectal). Thereafter, he had an intermittently high fever with chills until chemotherapy was started. On the 22d hospital day there was tenderness in the left upper quadrant. On the 25th hospital day he had pain in the right forearm over the tendon of the flexor digitorum without signs of inflammation.

When the blood cultures were found to be positive, treatment with sulfapyridine was started. After a period of 8 days, during which time some doses were omitted or reduced because of toxic symptoms, he was able to tolerate alternating doses of 1 gm. and 0.5 gm. of sulfapyridine every 3 hours. After the institution of chemotherapy there was a reduction of fever to a daily maximum of 99° to 99.5° (oral) until the 36th hospital day, when it rose to 102.2°. It remained at approximately this level for 48 hours. Then sulfapyridine was discontinued. The temperature gradually subsided to normal in the next 2 days and remained normal thereafter. The patient has remained in his usual state of health for 16 months.

Case 2.—J. T., a 29-year-old white nurse, admitted to the hospital on February 3, 1940, had been well until October, 1939, when she developed anorexia, fatigue, a mild dry evening cough, and drenching nightsweats. She continued her nursing duties until 6 weeks later, when she had a sudden attack of severe epigastric and right upper quadrant pain with vomiting and persistent nausea. After a week's rest in bed she returned to work, but complained of increasing fatigue, transient arthralgias, and evening temperatures ranging between 99.2° and 100.8°. Within the last month she had also had an attack of left-sided pleurisy lasting a few hours, another "gallbladder" attack, and an episode of pain, redness, and swelling in the left ankle which had subsided after a few days without residual. Since the onset of her illness she had lost 13 pounds.

She gave no history of dental extraction or infection shortly prior to her illness. No disturbance of the menstrual cycle was reported. She had been in the habit of expressing the contents of small acne pustules on the face.

She had had frequent tonsillitis and "growing pains" as a child. Her tonsils had been removed at the age of 14 "because of a bad heart." In 1936, at a preëmployment examination, she was told that she had rheumatic heart disease with aortic insufficiency and the examiner at that time remarked on the presence of clubbed fingers.

Physical Examination. The patient appeared pallid and chronically ill. Acne vulgaris was present over the face and shoulders. The left border of cardiac dullness was 9.5 cm. to the left of the mid-sternum. At the cardiac apex there was a systolic thrill and a loud, rough systolic murmur. The diminished aortic second sound was succeeded by a high-pitched, blowing, early diastolic murmur. The pulse rate was 110, regular, and of the Corrigan type. Her systolic blood pressure was 80 mm. Hg; diastolic, 46. The liver edge was palpable, 5 cm. below the costal margin in the midclavicular line on moderate inspiration. The spleen could not be felt. There was definite clubbing of the fingers. No petechiæ were noted. Pelvic examination revealed no abnormality.

Laboratory Data. All urine examinations were negative. Hemoglobin was 60% (Sahli); red blood cell count was 3,300,000 per c.mm.; and the white blood cell count was 12,550 with 76% neutrophils. The Kahn test on the blood serum was negative.

Three successive blood cultures showed up to approximately 150 colonies per cc. of *Staph. albus.* The orthodiagram revealed generalized cardiac enlargement. An electrocardiogram showed slight left axis deviation.

Treatment with sulfamethylthiazole, 1 gm. every 6 hours, was commenced on the 6th day of hospitalization. In 2 days the temperature had fallen to

normal from its previous range of 100° to 103° (oral), and it remained so for the remaining 20 days of hospitalization. The white blood count continued between 10,000 and 13,000. Blood cultures became decreasingly positive, and on the 12th, 14th, and 16th days after the commencement of the therapy they showed no growth. A final blood culture, taken 5 days later, was positive.

Throughout the period of observation the patient complained of transitory pains under the nails, but no petechiæ were seen. On the 18th hospital day a small, red, painful area appeared at the base of the big toe. On that day was started continuous intravenous administration of heparin. In 18 hours she received 44,000 units of heparin, and her clotting time by the method of Lee and White had risen from 13 minutes to 2 hours, 25 minutes. At this time she had a severe epistaxis, became very nauseated and vomited about 75 cc. of coffee-ground material. At about the same time she began to complain of a severe generalized headache and numbness of the hands. All treatment was stopped immediately. The headache persisted, the numbness became localized in the left hand which showed motor incoördination. She remained much prostrated and died suddenly in the night, 1 week after heparin had been discontinued.

Autopsy. The heart weighed 490 gm., and the left ventricle was hypertrophied. Microscopic examination revealed some interstitial myocardial fibrosis, but no typical Aschoff bodies. There were a few vegetations on the mitral valve which was not greatly deformed. The aortic leaflets were thickened, adherent, and covered by vegetations. The largest lesion was on the valve cusp beneath the left coronary ostium, and measured 5 mm. in diameter. A smear and a culture from the center of the vegetations showed an abundance of *Staph. albus*.

In the spleen were three small infarcts. In the fundus uteri were some small remnants of hyalinized placenta, which were not infected. There was a recent thrombosis in the large veins of the broad ligaments.

On the middle portion of the superior aspect of the right cerebral hemisphere there was an area of old hemorrhage, measuring 6 by 8 cm., in the subarachnoid and penetrating the superficial layers of the cortex, within which a zone of fresher hemorrhage about 1.5 cm. in diameter could be seen. Microscopic examination revealed an organizing embolus in a large superficial artery with fresh hemorrhagic necrosis in the center of the infarcted area and older, ischemic necrosis and reactive gliosis about the periphery of it. This was interpreted as evidence of a previous infarct, several days old, undergoing necrosis, into which a fresh hemorrhage had occurred.

Comment. The diagnosis of subacute bacterial endocarditis in Case 1 was fairly well established by the two positive blood cultures on consecutive days, the pleuritic pain without development of demonstrable pulmonary lesions (the congenital heart lesion would permit pulmonary embolism), transient microscopic hematuria and episodes of abdominal pain and tenderness. It is probable that the early treatment, 1 month after the first fatigue symptoms, perhaps before organisms were buried deeply in vegetations, was a factor in the favorable result. In Case 2, the persistence in vegetations of organisms of low-grade virulence and good susceptiblity to chemotherapy (*Staph. albus*) when the temperature had been normal for 3 weeks and 3 blood cultures had been negative, illustrates the difficulty in the chemotherapy of subacute bacterial endocarditis.

Temporary sterilization of the blood stream cannot be expected

to eradicate the infection localized in the vegetations and in infected emboli. The absence of relapse in Case 1 after chemotherapy had been discontinued because of drug fever may have been due to lack of time for large enough vegetations to form to protect the organisms from the sulfapyridine. It seems probable that chemotherapy should be continued for a long time, in most cases, in order to destroy all of the organisms in the vegetations.

We have not obtained, even temporarily, sterilization of the blood as frequently as has happened in most reported series. As more sulfonamide drugs become available it will probably be desirable to select the drug on the basis of the bacteriostasis demonstrable *in vitro* for the strain of organism cultured from the individual case. Swain[7] reported a marked variability in the susceptibility to sulfapyridine of four strains of *Strep. viridans* isolated from cases of subacute bacterial endocarditis. There was good bacteriostasis of two strains from patients whose blood streams became sterile and no bacteriostasis was demonstrated with two strains from patients whose bloods were not sterilized by treatment.

Kelson and White,[5] in their original report, recognized the danger of excessive bleeding incident to embolism in the treatment of subacute bacterial endocarditis with heparin. They reported one case who died with large occipital and subdural hematomas. These were considered to be embolic but it was thought that the heparin probably increased the hemorrhage. Other cases dying with cerebral hemorrhage during heparin treatment have been reported by Friedman, Hamburger and Katz,[3] Witts[9] and by Fletcher.[2] The latter noted that in all of these cases sulfapyridine had failed to sterilize the blood stream. He suggested that the persistence of a positive blood culture, and in particular the continued appearance of embolic signs, should contraindicate heparin treatment. Kelson and White[5] had advised beginning heparin administration from 4 to 7 days after sulfapyridine had been started. If a sterile blood stream is to be a prerequisite for heparin treatment, some method such as that proposed by Janeway[4] should be used to avoid the falsely negative blood cultures which we and others have obtained. In our Case 2, the continuously normal temperature obtained suggests that the blood cultures were truly negative but that this did not prevent the occurrence of emboli. Four other patients in this series, whose blood was not sterilized, received continuous intravenous infusion of heparin during 10- to 15-day periods, without accident. In Case 2, more heparin was used and the clotting time was prolonged more than advised by Kelson and White.[5] We have found considerable individual and day-to-day variation in the effect of heparin on clotting time. It had required considerably larger amounts of the same lot of heparin in a previous patient to prolong the clotting time to the desired value.

Staph. albus as the infecting agent in subacute bacterial endocarditis is uncommon, occurring in 4 of Thayer's[8] series of 362 cases. There has been one other case due to this organism in this hospital in the last 15 years. In one of Thayer's cases, the origin was ascribed to a paronychia. In our case, possible sources were traumatized acne postules or previous uterine infection.

Summary and Conclusions. Among 36 cases of subacute bacterial endocarditis treated with sulfonamide drugs there were 5 cases with one or more probably falsely negative blood cultures, 3 instances of probable sterilization of the blood stream with normal temperature temporarily and 1 cure of a probably proven case. Among 5 cases who also received continuous intravenous infusion of heparin there was 1 death with cerebral hemorrhage and no cure.

The reported experiences in the chemotherapy of subacute bacterial endocarditis warrant persistence in the accumulation of data concerning the different adjuncts to chemotherapy and the selection of a sulfonamide drug on the basis of bacteriostasis, demonstrable *in vitro*, for the strain of organism obtained from the individual case.

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THE TREATMENT OF TULAREMIA WITH ACRIFLAVINE.

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The treatment of tularemia continues to be very uncertain in spite of reports on successfully treated cases appearing in the literature from time to time. Perret,[11] reporting a study of 69 cases, is of the opinion that the treatment of this disease "is symptomatic." Since the introduction of sulfanilamide and its various derivatives, an increasing number of cases, treated with these drugs, is gradually appearing in the literature. Certain it is that even at the present time many types of therapy continue to be used, and these usually with most uncertain results.

Fisher[7] reported successfully on the use of neoarsphenamine in the treatment of 4 cases. Most writers, however, feel this drug to be ineffective and have accordingly discontinued its use. Early in

1932, Foshay[8a] first reported on a serum treatment for this disease. This was followed by a number of other contributions[8b–e] on the same subject, the latest being a statistical study of 600 cases in which serum treatment[8f] was used. His early remarks as regards these 600 cases are that "the results of serum treatment varied considerably, especially with relation to the clinical type that was presented, the presence or absence of extensive visceral lesions, the quantity and potency of serum that was given, and the stage of disease at which patients were treated." In a further analysis of these cases Foshay states that "statistical studies confirmed and, in some respects, amplified the clinical evidence that serum therapy modified favorably the course of the disease with respect to morbidity and to mortality."

Baer[1] reported the early use of small doses of Roentgen rays to the primary lesion in 2 cases resulting in the arrest of the progress of the disease. Bate,[4] in 1934, used the blood of a patient who had recovered from tularemia, giving it as a transfusion, with gradual improvement. Elson,[6] using the syrup of ferrous iodide, reported rather enthusiastically on 2 cases. He feels "that ferrous iodide is a specific in tularemia." Barthelme[3] has used metaphen successfully in the treatment of this disease, reporting 60 cases with 1 death. He reports that Shelton has used quinine successfully.

Sulfanilamide and its derivatives have received much attention in the past 3 years in the treatment of tularemia. Miller and Bannick,[10] reporting a case, used heavy doses of sulfanilamide over a period of 6 days with apparently no benefit. This patient showed a "prompt improvement" following the use of 2 doses of Foshay's antitularemia serum. Powers and Powers[12] later reported recovery of a case following the use of sulfanilamide and Foshay's specific antiserum. Curtis[5a] reported the successful treatment of a case with sulfanilamide; and this was followed by a report[5b] of 4 additional cases similarly treated. Weilbaecher and Moss[13] report 5 interesting cases of tularemia treated with sulfapyridine, sulfanilamide, sulfamethylthiazole and sulfathiazole. Their observations were such as to show benefits only with sulfamethylthiazole and sulfathiazole. Johnston reported a very unusual case in which "rapid clinical improvement ensued immediately following the use of sulfanilamide." However, "during the subsequent rise in fever the drug was purposely withheld, in spite of which a prompt drop to normal occurred." From this observation he inferred that "one cannot know whether sulfanilamide was of any value or not." Barkley,[2] after using large doses of sulfanilamide for 5 days without any results, changed to sulfathiazole in large doses for 4 days with a clearing up of symptoms 48 hours after the latter drug was started.

From the above reports it is readily apparent that the treatment of this disease is still very uncertain. Since December 27, 1940, the writer has had occasion to treat 3 cases of tularemia. One of these

cases was acute and the other 2 definitely chronic. Because of an interesting experience with acriflavine in the treatment of a case of typhus in May of 1940, I decided to use this drug on these cases. It is my intention in this report, therefore, merely to present the histories of these 3 cases of tularemia in which this drug was used apparently with distinct benefit. Although I do not make any claims for any form of therapy on the basis of 3 cases, nevertheless, the results obtained, I believe, adequately justify the presentation of this subject, in the hope that additional investigations might determine its true value.

Case Reports. CASE 1.—Mr. N. F., aged 21, a worker in the fish and game market, became ill on Christmas Day, 1940. He had returned home late from a Christmas Eve party feeling well. When awakened at 3 in the afternoon, he had a headache, and felt badly. From this time until December 29, when he first noticed he was in a hospital, he remembered nothing at all. A history from the family showed he had been skinning rabbits for 2 months, the last being on December 24. His temperature was slightly over 105°. He was incoöperative and talked incoherently. Examination of his hands showed a small ulcer about 3 mm. in diameter on the dorsum of his right index finger over the distal interphalangeal joint. The right axillary lymph nodes were enlarged, very tender and painful. A diagnosis of tularemia was made and he was sent to the hospital. An agglutination test for tularemia on January 2 showed "a few clumps in a dilution of 1 to 20." On January 7, the test was positive at 1 to 320.

Until January 3, he was treated symptomatically. Sulfathiazole and neoarsphenamine were next used. On January 11, he was given 100 mg. of acriflavine, dissolved in 250 cc. of normal saline, intravenously. This was followed by 1000 cc. of 5% glucose in normal saline as an infusion. In the afternoon of this day the patient said he felt "fine." His temperature for the first time failed to go above 100° F. The next day there was a very marked improvement in his clinical condition. He volunteered the information that he was feeling fine for the first time since becoming ill. His pains were all gone. The enlarged and painful right axillary lymph nodes seemed to suddenly become painless. The maximum temperature on January 12 was 99.6°. By noon of this day it dropped to 99°, reaching this level again the following morning and at 8 P.M. on January 14. During the afternoon of this latter day, another 100 mg. of acriflavine was given. The next morning the temperature was 98 and never again went above normal. His strength seemed to return so rapidly, and he felt so greatly improved that he wanted to go home the next day. He left the hospital on January 19.

On February 11, the patient felt strong and entirely recovered from his illness. He had gained about 10 pounds; and the enlarged axillary lymph nodes were still painless and seemed to be getting smaller. The region of his primary lesion showed a darkly pigmented, small scar.

CASE 2.—Mr. J. M., aged 27, a market worker, had been cleaning rabbits since the season started. On the morning of December 11, 1940, he awakened with chills and fever. All day he felt chilly at work and that night began having pains all over his body. Continuing to feel "miserably," and now having pain in right axilla, he called in a physician on December 13, who sent him to the Charity Hospital with the diagnosis of "rabbit fever." The primary lesion was found on the volar surface of his right index finger, over the distal interphalangeal joint. The agglutination test for tularemia was positive (1 to 320) on December 28. He was treated with large doses of sulfathiazole and on December 30 was permitted to go home.

Upon leaving the hospital he felt badly and very weak; which continued for many days. At times he felt fairly well, but usually he was tired, lazy, droopy and restless. He was never able to return to work.

On January 23 a painful tooth was extracted. The following day he again developed high fever, with pains over his entire body, free sweating and had several chills. The right axillary mass became more painful, and after several days of self-medication he called me in (January 27), and I sent him to the hospital. The agglutination test for tularemia was reported as positive.

On January 30 acriflavine was given, as in Case 1. The next day the fever dropped to normal and remained so for 2 days, when, following another intravenous administration of acriflavine, it went to 100° for a short time. After this it never went higher than 99.4° F., which followed a third treatment with acriflavine on February 5. He was permitted to go home on the following day.

After the first administration of acriflavine, the patient said he felt markedly improved. His malaise disappeared completely and his strength seemed to return overnight. His appetite became very good, and he frequently asked for second servings. He showed signs of a returning ambition and was anxious to go back to work. His general appearance markedly improved, and pallor was soon replaced by a rosy complexion. When seen in the office 4 days after leaving the hospital he said he felt "a thousand times better." The pain in his right axilla had disappeared after the first administration of acriflavine. On February 27 he was working and feeling fine. He had regained much of the weight he had lost, and had never had any more trouble.

Case 3.—Mr. A. B., aged 21, began cleaning rabbits in large numbers, when the season started, with the above Mr. J. M. At about 2 A.M. on November 26, 1940, the patient was awakened by a chilly feeling and continued having chills all night. In spite of feeling very badly, he forced himself to go to work. Although he felt much depressed and uncomfortable, he had no pains. The condition was diagnosed and treated as influenza, but as he was no better by November 30, he went to the Charity Hospital. On November 29 he first noticed a small painless mass in his left axilla.

At the hospital a small lesion, that had been present several days, was discovered proximal to the nail base on the dorsal aspect of the left middle finger. A diagnosis of tularemia, ulcero-glandular type, was made and he was admitted. The agglutination test for tularemia was positive. Here he was treated with large doses of sulfathiazole. He was discharged on December 29.

On leaving the hospital he continued to feel badly and had an occasional chill. His family physician incised and drained much pus from large left epitrochlear and left axillary lymph nodes (about February 19). He continued running a low-grade fever.

I first saw him on March 10, and sent him to the hospital on the following day. During his 12 days there he was given 4 intravenous treatments of acriflavine administration in the same manner as for the above 2 cases—the administrations being given every 72 hours. Following these treatments he said he felt much stronger and his appetite returned. Although he continued to run a low-grade fever at irregular intervals, he was permitted to go home, in the hope that the fever would soon subside entirely, and he would recover fully. In spite of the moderate clinical improvement, however, he continued to run this low-grade fever; and on April 7, a check-up examination showed a moderately enlarged liver and a definitely palpable spleen. The epitrochlear sinus had stopped discharging in the hospital, but the axillary sinus continued draining slightly. On April 9, as the spleen seemed larger, he was referred to Drs. Menville and Ané, who subjected

his spleen to 4 Roentgen ray treatments. The organ diminished in size; an epigastric discomfort passed off; and in spite of some discomfort after each treatment, an apparent improvement was admitted by the patient.

Despite this, however, he continued running fever, and by April 14, the fever began going over 100° F. Foshay's serum was considered, but the cost could not be afforded. I then decided to keep him at absolute rest in bed, treating him symptomatically at his home. After nearly 10 days as no improvement was observed, I decided he should reënter the hospital for more intensive treatment with acriflavine.

On April 28 he was admitted to the French Hospital, where he remained until May 18. Another agglutination test for tularemia was reported positive. During this time he was given 8 intravenous administrations of acriflavine according to the usual method (April 29 and 30, May 1, 3, 5, 7, 10 and 14). After his second treatment he said he felt very much better. His strength seemed to return, his appetite improved, and he was able to rest better. Each administration of acriflavine was followed by a slight rise of not more than 1° of fever, except the last, which did not show any rise in temperature at all above normal. The fever gradually dropped and after May 12, no more fever was observed. His clinical improvement was marked: there was no more malaise, the appetite almost became voracious, he no longer felt droopy and had no more chilly sensations. His strength seemed to return so rapidly that he was soon asking permission to return to work. On reëntering the French Hospital he weighed 132 pounds, and on July 1 his weight was 138¾ pounds. At this time he said he had never had any more trouble.

Summary. The 3 cases herein reported have continued to be observed from time to time. Each is well, has picked up weight, feels fine and is back at his work. In each instance it appeared that the administration of acriflavine was followed by a definite clinical improvement in the patient's condition. In the first case the fever likewise seemed to decline very definitely, following the use of this drug; whereas, the other 2 cases—although showing a definite tendency to a lessened fever—did not appear to be as definitely affected in this respect. The latter cases, however, were chronic in type when seen by me. Each sought relief from a recrudescent attack; and the third one was the most protracted case, resistant to several forms of therapy and very stubborn to every form of treatment, including the first series of acriflavine administrations. In this third case the disease had become deep seated and very probably involved many of the hidden lymph nodes. Because of this probable extensive involvement of the lymphatic system, the illness was protracted and stubborn in its response to all the forms of therapy used.

In no case was any disagreeable reaction observed from the use of acriflavine. Actually, aside from an occasional slight rise of temperature, following the administration of this drug, no untoward response was at any time seen. The writer feels that—if the drug was actually of any benefit—it probably acted as a bacteriostat. As such, the acriflavine very probably inhibited the growth of the organisms sufficiently to permit the host enough time to increase his antibodies to the point of finally overcoming the infection. In each case, it appears that the body defense did not become definitely

well established until acriflavine was administered. In the third case the drug had eventually to be given more frequently in order to have a definite inhibitory influence on the growth of the offending organism.

Conclusions. 1. The treatment of tularemia is briefly reviewed.

2. Though the writer has had no experience with the use of Foshay's antitularemia serum, the results from its use, as reported by Foshay himself, do not seem to have been sufficiently impressive to warrant its routine employment in all cases.

3. Sulfanilamide and its derivatives are of little, and probably of no value in the treatment of this disease.

4. Histories of 3 cases of tularemia are herein presented. The use of acriflavine intravenously seems to have been of distinct benefit in the treatment of each. The writer agrees that further observations in the use of this drug in the treatment of tularemia are necessary before making any definite evaluation of its efficacy.

5. Should acriflavine prove to be of distinct benefit in the treatment of tularemia, it will very probably be shown that its action is bacteriostatic, permitting the host time to build his defense against this infection.

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THE LOCAL TREATMENT OF PYOGENIC CUTANEOUS INFECTIONS WITH SULFATHIAZOLE IN AN EMULSION BASE.*

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Infectious dermatoses of various types offer an inviting field for treatment by the local application of sulfonamide compounds. In

* This study was aided by a grant from the Smith, Kline & French laboratories.

many minor dermatologic conditions the risk of reactions following the administration of sulfonamides by mouth is unjustified, and the present methods of local treatment of superficial skin infections are not entirely satisfactory.

This study is an attempt to determine: 1, the effectiveness of local sulfathiazole therapy in the treatment of various dermatologic lesions caused in whole or in part by pyococci; 2, the most efficient vehicle of application; 3, the evidences of local or general toxicity incident to such therapy; and 4, the effect on the various bacteria found in such lesions. A total of 190 patients has been treated.

The recorded studies concerning the local application of sulfonamide compounds in superficial dermatologic infections are as yet few in number, and some of them do not include detailed data. Jaeger[7] first applied Prontosil to various dermatoses in a vehicle of equal parts of acetone and alcohol, and in cholesterinized petrolatum. He noted no irritation at the site of application, and reported good results in infectious skin diseases, sweat gland abscesses, dermatomycoses, infected wounds, lupus vulgaris, and burns. Occasional improvement was noted in cases of eczema. Merz[14] used 10% sulfanilamide ointment in acne, furunculosis, and impetigo, occasionally combining it with oral or intramuscular administration. Lamers[11] treated herpes zoster with an acetone-alcohol tincture of Prontosil, with "invariably good results." Other studies include those of Dey,[2] in which he applied sulfanilamide powder in 20 cases of boils, ulcers, and other mild skin infections, and Smith,[18] in which 5 cases of infected ulcers and burns were treated by soluble Prontosil in compresses or lanolin ointment.

Veal and Klepser[20] treated 140 patients suffering from pyogenically infected wounds, with sulfanilamide powder and an ointment containing 10% sulfanilamide, 2% allantoin, and 0.5% chlorbutanol in a glycerinated stearic acid ointment. They noted early inhibition of bacterial growth, with sterilization of the wound in about 8 days. It was found that the ointment form seemed to promote granulation tissue, and initial application of the powder for from 4 to 6 days, followed by use of the ointment, was suggested. Guyton[5] found that the use of sulfanilamide in ointment had no striking effect attributable to sulfanilamide alone in catarrhal or gonorrheal conjunctivitis, or in trachoma. The results in corneal ulcers were good. Solutions of sulfanilamide were found to be less effective than ointments, and the details of preparation of a suitable base are given. The base employed was apparently of a water-in-oil emulsion type. The sodium salt of sulfapyridine was found to be irritating, apparently because of its higher pH.

The most extensive studies of local sulfonamide therapy have been concerned with its use in surgical conditions, particularly infected wounds,[6] after clean operative surgery,[9] and in compound fractures.[8] The references given are representative, since restric-

tions of space do not permit of a complete résumé, but there is adequate convincing evidence that the local application of sulfonamide compounds in tissue is an effective bacteriostatic agent.

Vehicle of Application. Application of sulfonamides to the skin by dusting the powdered drug on the affected site has definite disadvantages in dermatologic therapy. The powder does not remain in contact with the site of infection unless an occlusive dressing is applied, and is easily washed away by serous or purulent secretion. It tends to become conglomerate and not to remain evenly distributed over the area of application. An effective vehicle should, we believe, accomplish the following: 1, retain the sulfonamide in a finely divided state at the site of application for a reasonable period; 2, permit close contact of the compound with the site of infection; 3, be miscible with serous and purulent drainage; 4, not form an impermeable and inert covering under which bacteria might grow readily; and 5, permit easy removal of bacteria contained in crusts and surface débris. In previous studies we have attempted to determine the best vehicle of application for certain of the commonly used skin antiseptics,[12] and have observed the effect of various ointment bases and antiseptics on the bacterial flora of the normal skin.[16] Applying the criteria listed above to various ointment bases in common use, it seems to us that most of them have certain disadvantages. The all-grease bases, such as petrolatum, lanolin, or simple ointment are effective in retaining a medicament at the site of application, but may not allow intimate contact of the antiseptic agent with the site of infection, do not mix easily with exudate, are sometimes removed with difficulty, and probably increase the growth of bacteria under the grease film. Substances such as pectin, tragacanth, and bentonite are useful, but dry and flake off quickly unless a considerable amount of an anti-drying agent is added. Lotions have a limited usefulness. On theoretical grounds, water-in-oil emulsions may not show any superiority over grease bases, especially if the active ingredient is contained in the external oil phase. An oil-in-water emulsion type base, in which the sulfonamide is first suspended or dissolved in the external aqueous phase, offers certain advantages in that it theoretically allows evenly distributed contact of the antiseptic with the site of infection, has a low surface tension, mixes readily with exudates, and is easily removed by washing.

Of the available sulfonamides we have employed all of the following to some extent, sulfanilamide, sodium sulfadiazine, and sulfathiazole. However, the bulk of our experience has been with sulfathiazole. It has the advantage of being relatively effective against both staphylococci and streptococci. It is little soluble, and the probability of significant absorption is not great. The sodium salts of the various sulfonamides have a possible advantage of greater

solubility, but have a relatively high pH and may be more irritating when applied locally.

One hundred and ninety patients with various dermatoses have been treated with sulfathiazole applied locally in ointment form. Of these, in 134 it seemed probable that pyococci were solely or partially responsible for the eruption noted. It has been found convenient to divide these cases into the following groups, especially since the dermatologic terms applied to various superficial and deep pyodermas are numerous, confusing and often meaningless.

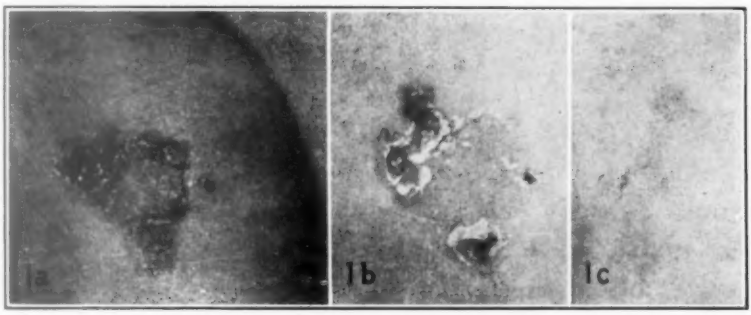

FIG. 1.—*a*, Staphylococcic impetigo of chin unimproved after treatment with ammoniated mercury ointment for 1 week. *b*, After 3 days of treatment with sulfathiazole in emulsion type base. *c*, After 5 days of treatment.

Group I includes all cases in which a pyogenic infection was apparently the primary cause of the skin changes. These were all acute cases of from a few days to 1 month in duration and included streptococcic and staphylococcic impetigo, infectious eczematoid dermatitis, Bockhardt's follicular impetigo, and superficial ecthyma. The lesions were superficial in character, not ordinarily followed by scarring, and characterized by pustules and seropurulent crusts (Fig. 1 *A–C*).

Group II includes cases of chronic eczematoid dermatitis in which pyogenic infection was at least a partial etiologic factor. Often there were no frank discrete pustules, but simply diffuse erythema, scaling, maceration, and fissures. These conditions are frequently diagnosed on morphologic grounds as fungous or Monilia infections. Included are acute intertrigo (behind ears, under breasts, in crural folds, gluteal cleft, and between toes), dermatitis repens, chronic infectious eczematoid dermatitis, and coccogenous sycosis. They are characterized by frequent contributory general medical or local factors, including obesity, diabetes, local vasomotor changes, hypersensitivity to bacterial or fungous antigens, and hyperidrosis (Fig. 2 *A*, *B*).

Group III includes chronic deep ulcers apparently caused by pyococci. Such lesions are caused by a variety of microörganisms, produce varying degrees of scarring, and are also frequently associated with important contributory local or general factors, including vascular disturbances, trauma, anemia, and diabetes. In this series we did not encounter a case which we felt corresponded to the chronic symbiotic infections described by Meleney[13] or the phagedenic ulcers recently discussed by Greenbaum[4] (Fig. 3 *A*, *B*).

Group IV includes acute or chronic pyodermia secondary to eczema, fungous infections, dermatitis venenata, seborrheic dermatitis, herpes or

injury. At times the secondary infection was acute and overt, with pustules, erythema, pain, lymphangitis, and occasional evidence of general reaction. This was particularly true in dermatitis venenata or fungous

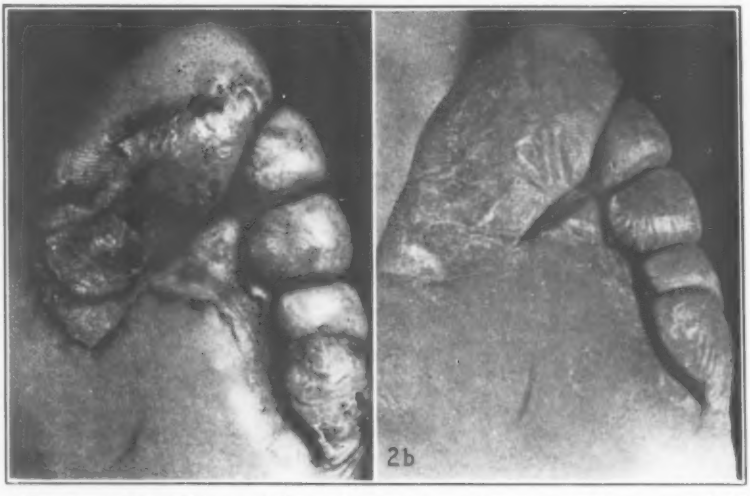

FIG. 2.—*a*, Intertriginous inflammatory eczema of toes suggesting a superficial fungous infection. No fungi were found on repeated culture, hemolytic streptococci isolated in pure culture. *b*, After 1 week of treatment with sulfathiazole emulsion ointment. In this patient one foot remained clear and the other showed a recurrence which was not controlled by sulfathiazole ointment.

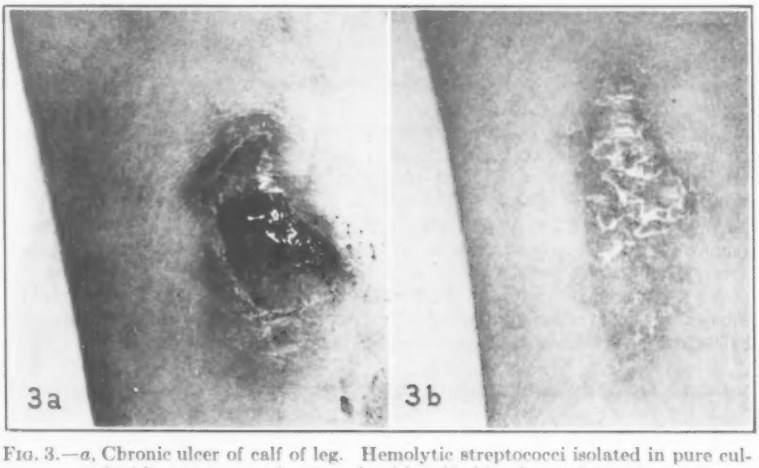

FIG. 3.—*a*, Chronic ulcer of calf of leg. Hemolytic streptococci isolated in pure culture. *b*, After treatment for 1 week with sulfathiazole emulsion ointment.

infections of the feet infected with streptococci. In secondarily infected eczema or seborrheic dermatitis, however, there may be little evidence of a local purulent reaction, and yet apparently pathogenic microörganisms may

be repeatedly recovered over a long period of observation. In our experience, acute flare-ups of ringworm of the feet are more often due to pyogenic invaders than to the fungi themselves.

FIG. 4.—*a*, Acute streptococcic pyoderma complicating chronic intertriginous fungous infection of toes. Associated femoral adenopathy. *b*, After treatment for 2 days with sulfathiazole emulsion type ointment. The chronic underlying fungous infection was not affected.

In addition we have treated 30 patients suffering from various miscellaneous dermatoses, including dermatitis and eczema, dermatophytids and "pyids," cellulitis and erythema multiforme. In some of these, occasional good results follow sulfonamide therapy by mouth. Nine patients with folliculitis or furunculosis were treated, and 13 patients with acne.

The types of bases used in this study were as follows:

Grease base:	
Sulfathiazole	5.0%
Simple ointment (U.S.P.)	95.0%
"Vanishing" type stearate base:	
Sulfathiazole	5.0%
Carbitol	10.0%
Stearic acid	20.0%
Peanut oil	4.0%
Potassium hydroxide	1.0%
Water*	60.0%
Pectin base:	
Sulfathiazole	5.0%
Pectin	10.0%
Benzoic acid	0.2%
Ringer's solution*	84.8%
Oil-in-water emulsion type base:	
Sulfathiazole	5.0%
Sodium lauryl sulphate	1.0%
Stearyl alcohol	10.0%
Cetyl alcohol	3.0%
Spermaceti	10.0%
Glycerine	10.0%
Water*	61.0%

* Glycerine may be added for its anti-drying action without impairing the action of sulfathiazole. Dr. R. H. Blythe prepared the ointments used in this study.

Clinical Results. In Figure 5 are given the results of treatment in 134 patients with dermatoses due in whole or in part to pyococci.

By 100% improvement is meant a rapid and complete return of the skin to its normal state. The rapidity of response to local sulfathiazole therapy, when it is to prove effective, surpasses that seen with any other local antiseptic, in our experience. Cases classified as showing 75% improvement responded rapidly to local sulfathiazole therapy, with relief of symptoms, but did not progress to com-

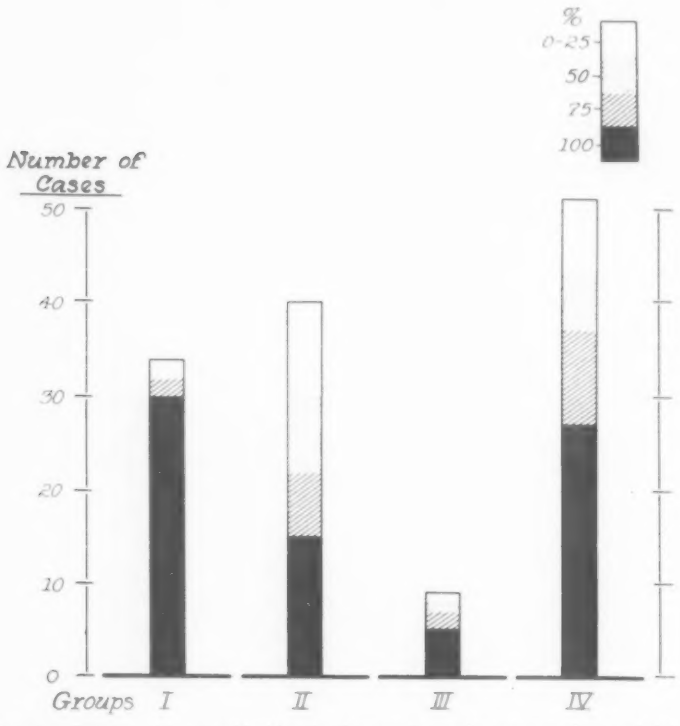

FIG. 5.—Results of local sulfathiazole therapy in various types of superficial dermatoses associated with infection.

Group I—Primary pyoderma (impetigo, acute infectious eczematoid dermatitis, and so on).

Group II—Chronic eczematoid and intertriginous lesions of varied etiology, including pyogenic infection.

Group III—Deep chronic ulcers of skin and subcutaneous tissues.

Group IV—Secondary pyogenic infections of preëxistent dermatoses (including dermatitis venenata, fungous infections, and eczema).

plete cure, and other measures became necessary. A 50% improvement indicates definite lessening of objective and subjective signs and symptoms of infections, and could be classed as "satisfactory." Improvement under 50% was partial and often temporary and could be duplicated or surpassed by a number of other local methods of treatment.

It is noted that in primary superficial pyodermias, rapid, complete cure was obtained in 30 of 35 cases treated. In such cases the

changes were easily determinable in 48 hours, often in 24. Streptococcic impetigo responded most rapidly, with absolute prevention of new lesions as a rule, and complete disappearance of all lesions in 4 to 5 days.

In the group of processes of chronic infectious eczematoid character (Group II), the results were less striking, 15 of the 40 cases showing a rapid and complete cure, 7 a 75% improvement, and 8 a 50% improvement. Nevertheless, because these cases are ordinarily very resistant to other methods of treatment in our experience, local sulfathiazole is regarded as being of distinct value.

In 9 cases of relatively deep chronic ulcers of the skin and subcutaneous tissues, complete rapid healing was noted in 5 (Fig. 3 *A*, *B*). In such cases it was ordinarily convenient to pack the ulcer with sulfathiazole emulsion and apply a dressing.

In Group IV, in which the infection was obviously a complication of a preëxistent dermatosis of chronic or acute character, complete cure, with return of the skin to a normal state within 1 week, was noted in 27 of 51 patients. No irritation from the sulfathiazole ointment was noted in patients suffering from a previous dermatitis venenata. There was no indication that sulfathiazole ointment had any effect on an underlying chronic fungous infection or seborrheic dermatitis, and only occasionally did a preëxistent eczema show permanent improvement.

An illustrative case in this group is the following:

Case 1.—J. R., a white girl, aged 9, sustained a dermatitis venenata due to rhus, on the legs, arms, and face. Five days after the dermatitis appeared, thin-walled superficial pustules on a highly inflammatory base were noted (typical of streptococcic pyoderma). These increased in extent, affecting principally the previously dermatitic skin. Femoral, axillary, and cervical lymphadenopathy appeared, and the fever rose to 104°. A pure culture of hemolytic streptococci was obtained. The pustules were all opened, tissue débris and crusts removed, and sulfathiazole emulsion ointment applied every 3 hours. The temperature became normal in 24 hours, and remained so. Complete healing occurred within 4 days. In another patient with an entirely similar eruption, sulfathiazole was also given by mouth, with similar prompt improvement.

In the miscellaneous group of 30 cases of dermatitis, eczema, "ids," low-grade cellulitis, and erythema multiforme, no improvement was noted in 17, and the moderate improvement noted in the remaining 13 cases was no more than would have been expected from application of a bland ointment, or incidental to the normal course of the disease.

Our experience with deep follicular infections, either primary or secondary to acne vulgaris, is as yet limited. It is believed that sulfathiazole ointment has no effect on a developing unopened lesion. After surgical drainage, it is uncertain that improvement is more rapid with sulfathiazole ointment, but we have the impression that the development of secondary satellite lesions is impeded. The course of folliculitis, furunculosis and pustular acne vulgaris is

so variable that any conclusions must await experience in a large number of cases. It was noted that the emulsion or stearate vanishing bases did not cause flare-ups of acne of the type sometimes noted after application of various greases.

Directions for Application of Ointment. In order that an adequate concentration of sulfathiazole may be maintained at the site of infection, and that the effect of substances interfering with its action (peptone, pus) may be counteracted, certain rules for application have been established. These are:

1. Application of the ointment four to six times daily, unless a closed dressing is used.

2. Careful cleansing of the affected area as vigorously as may be tolerated by the patient. Crusts and débris from vesicles and scales should be removed scrupulously. Saline, boric acid, or dilute potassium permanganate solution applied as compresses or soaks are useful in cleaning up lesions.*

3. Surgical drainage of all pustules or vesicles, even the smallest. *Sulfathiazole ointment has little effect when applied to the top of an intact pustule.* Patients or attendants may be trained to open pustules with clean manicure scissors safely and satisfactorily.

Bacterial Findings. In 119 of the 134 cases in Groups I, II, III and IV, of this series, bacterial cultures were performed. The technique will not be given in detail. It is important that cultures be incubated under both aërobic and anaërobic conditions.

It is often difficult to evaluate the significance of a particular strain of bacteria isolated from a lesion of the skin, particularly in chronic intertriginous or patchy eczematoid lesions. We record these bacterial findings as factual information, but shall not in this paper attempt an extensive review of the controversial opinions as to the pathogenicity of the organisms recovered. Since the original work of Sabouraud[17] on streptococcic dermatoses, several studies of the rôle of streptococci in various skin lesions have appeared, of which those of Mitchell,[15] Kinnear,[10] and Epstein[3] are representative examples. In several thousand scrubs of the normal skin of the hands and forearms of 8 subjects, using the Price technique, we have not encountered a single culture of streptococcus hemolyticus. This is not in accord with the statements of other observers.[21] The rôle of staphylococci in a particular skin lesion is even more difficult to determine, because they are regularly isolable from normal skin. We believe that some of the findings in cases in which bacterial cultures were done repeatedly during sulfathiazole therapy may shed light on the significance of the organisms recovered.

Figure 6 gives the bacterial findings in our cases, together with the corresponding number of cases showing a "satisfactory" improvement of over 50% of sulfathiazole therapy. The patients grouped under "streptococcus" or "staphylococcus" showed a pure

* Sulfathiazole in an emulsion type base may be used in place of soap. A study of its effect in reducing the bacterial flora of the normal skin is in progress.

or heavily predominant culture of the corresponding bacterium, always of hemolytic type. Under "mixed" infections are grouped those cases in which a large number of strains were obtained, without one clearly predominating. The strains included *Strep. viridans*, indifferent streptococci (short chain, non-hemolytic), *Staph. aureus* and *albus*, micrococci, sarcina lutea, and diphtheroids. It will be noted that when hemolytic streptococci were recovered as the predominant organism, some clinical improvement was always noted following sulfathiazole therapy. In cases in which hemolytic

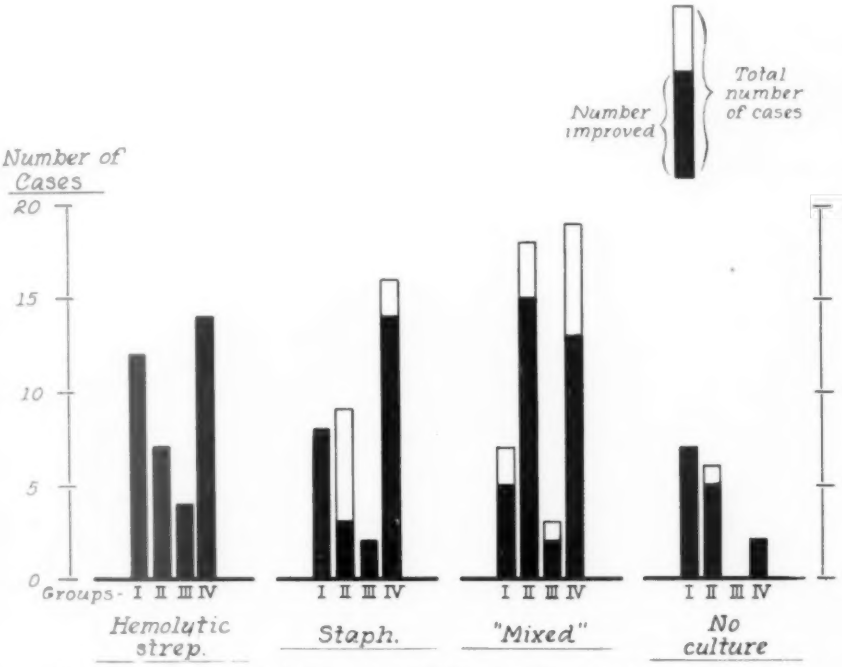

FIG. 6.—Results of bacteriologic culture in 119 cases. (The number of cases showing a satisfactory improvement of over 50% is indicated in black.)

staphylococci were recovered in pure or predominant culture, the clinical results were less striking, particularly in the group of chronic eczematoid dermatoses with evidence of infection. In the cases showing "mixed" cultures the results were likewise not as good as when streptococci were predominant.

The findings in the cases previously resistant to other types of therapy seem instructive (Fig. 7). These were principally patients with dermatoses of Groups II, III or IV, and the prolonged previous treatment included variously potassium permanganate soaks, ammoniated mercury ointment, Castellani's carbolfuchsin paint, gentian violet, acriflavine and Roentgen ray therapy. In such patients, when hemolytic streptococci were the sole or predominant organ-

isms, complete cure was obtained in 12 of 28 cases. When the culture revealed principally staphylococci, complete cure was noted in only 4 of 26 patients, and 10 were unimproved. These results might be interpreted in two ways, either sulfathiazole ointment is less effective against infectious skin lesions in which large numbers of staphylococci are found, or dermatoses associated with local staphylococcic infection are more readily influenced by standard treatment measures and sulfathiazole ointment offers less superiority.

Results in Cases of Superficial Infection Previously Resistant to Other Types of Treatment

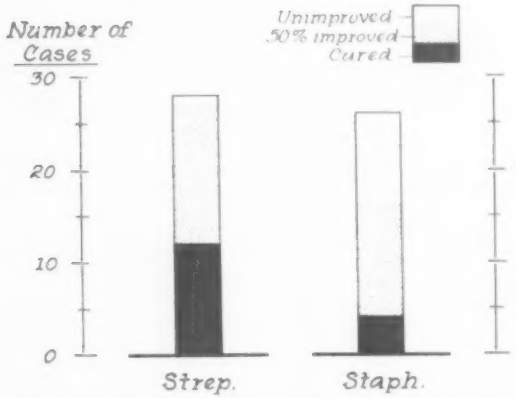

FIG. 7.—Local sulfathiazole therapy in patients previously resistant to various other types of treatment.

Comparative Effect of Various Bases. In our initial experience with all-grease bases, it was soon apparent that the clinical effect, while often surpassing the improvement obtained with ammoniated mercury ointment, was not as good as when the sulfathiazole was incorporated in an emulsion or stearate vanishing type base. After treatment of approximately 15 cases, all-grease bases were therefore discarded for a time, and our studies confined to the vanishing or emulsion type bases. More recently we have compared the effect of sulfathiazole in emulsion base and in a proprietary grease base apparently containing a mixture of petrolatum and lanolin. We have concluded that an all-grease vehicle for the application of sulfathiazole, while occasionally moderately effective in superficial pyodermas, has a slow and inefficient action when compared with either an emulsion or stearate type base. The following case is illustrative:

CASE 2.—R. B., a white male, aged 5, presented a widespread severe pyoderma with superficial pustular, bullous, and ecthymatous lesions distributed on the scalp, face, back, arms, and thighs. The initial lesion had been noted on the face 1 week previously, and the extension to other parts of the body had been rapid, with chills and fever for 2 days. Ammoniated

mercury ointment had been applied for 2 days prior to admission, without effect. Between July 9 and 16, 1941, a 5% ointment of sulfathiazole in petrolatum and anhydrous lanolin was applied three times daily. Slight improvement occurred, though new lesions developed. Treatment was then changed to 5% sulfathiazole in an emulsion type base, and complete healing of all lesions occurred in 2 days.

In general, stearate "vanishing" bases have produced a more satisfactory effect than did various grease bases. At times, improvement following application of this type of base was as rapid as with the emulsion base. However, two disadvantageous features were noted, one being that the vanishing ointment tended to dry more rapidly, and the other that patients frequently complained that the stearate base was less soothing.

Our experience with the pectin type base is limited. We detected no advantage therapeutically, and such an ointment tended to flake off rapidly.

In summarizing the effectiveness of the various bases as a means of producing a satisfactory therapeutic action of sulfathiazole on superficial dermatologic infections, the following rather arbitrary method of expression might be used. If the emulsion type base is regarded as, say, 90% efficient, we would place the effectiveness of the stearate type base at approximately 70%, and the grease base as 50% or less. These are pure estimates, rapidity of action on the infection being the first criterion, but with consideration of other factors such as the frequency of application necessary, the subjective sensations of the patient following its use, washability, and apparent penetration under crusts and débris to the site of infection.

Bacteriologic Findings in Treated Patients. In patients in whom the skin lesions did not heal within a few days, bacteriologic culture was done at weekly intervals. When large numbers of indifferent streptococci or *Strep. viridans* were found (11 cases), disappearance of these bacteria was noted within 2 weeks. In 23 cases in which hemolytic streptococci were predominant, in only 1 did the streptococci persist in hemolytic form. In 8 cases the streptococci disappeared entirely within 2 weeks. In 9 cases the organisms became indifferent (non-hemolytic, short chain) within 2 weeks, and disappeared entirely within 1 month. In the rest of these cases the streptococci became indifferent in varying periods of time and then disappeared, with the exception of the above noted case. In chronic eczematoid lesions, disappearance of streptococci was not always accompanied by clinical improvement. Hemolytic *Staph. aureus* persisted apparently unchanged in 90% of patients in whom it was the predominant organism recovered. In a few cases the staphylococci were apparently replaced by micrococci.

In general, *Strep. hemolyticus* tended to become indifferent in type and then disappear in lesions treated with sulfathiazole ointment. *Strep. viridans* and indifferent streptococci tended to disappear promptly. *Staph. aureus hemolyticus* tended to persist in treated lesions, regardless of the clinical course of the process.

Absorption of Sulfathiazole, Local and General Evidence of Toxicity. In most of the patients in whom a sulfathiazole ointment was used, the total amount of sulfathiazole applied to the skin was so small as to make the possibility of absorption of significant amounts unlikely. For instance, in the usual case of impetigo, not more than 30 gm. of the ointment were required daily, a total of 1.5 gm. of sulfathiazole. The largest amount required was in a patient with a generalized dermatitis from which hemolytic streptococci were repeatedly isolated, when 120 gm. of the ointment were required daily, a total of 6 gm. of sulfathiazole. In no patient were there any symptoms or signs indicative of sulfathiazole toxicity. In 40 patients under treatment with varying amounts of sulfathiazole ointment, determination of the blood sulfathiazole level was done according to the method of Sunderman and D. S. Pepper.[19] In no patient was any sulfathiazole detectable in the blood.* In 4 patients experimental introduction of sulfathiazole through the skin was attempted by the application of 180 gm. of the emulsion daily (9 gm. sulfathiazole). In 1 of these patients, in whom there were very large areas of dermatitis, a "barely detectable" trace of sulfathiazole (under 0.05 mg. per 100 cc.) was found in 2 of 12 determinations performed; in the rest no sulfathiazole was detectable.†

We have seen no indication that sulfathiazole ointment applied to one area of the body has any effect on lesions at another site, *i. e.*, that applied sulfathiazole acts by absorption into the blood stream. Its effects are purely local, at least in superficial dermatologic lesions. In interpreting this finding it should be kept in mind that there are included no patients in whom very large ulcers were packed with considerable amounts of sulfathiazole ointment. Under such conditions some absorption of sulfathiazole might be expected, but probably of an order less than would be the case with more soluble sulfonamide compounds. In our experience with superficial dermatologic lesions, local sulfathiazole therapy applied in all-grease, stearate or emulsion ointments is entirely free from any general reaction attributable to the sulfathiazole.

In a few patients with chronic eczematoid eruptions, flare-ups have been noted in conjunction with the use of sulfathiazole ointment, particularly when the stearate type base was used. In no patient was there any indication that the sulfathiazole *per se* was responsible, and no exacerbation was noted in a patient with a dermatosis primarily due to infection. The emulsion and stearate bases contain certain possible contact sensitizing agents, including particularly sodium lauryl sulphate and glycerine, but a considerable previous experience of two of us (D. M. P. and C. S. L.) with the various ingredients of these bases had not indicated that their sensitizing capacity is significantly high. Ordinary ammoniated mercury ointment, even in strengths of 1%, has a far higher sensitizing

* The blood sulfathiazole determinations were done by Dr. F. W. Sunderman.
† This portion of the study was carried out by Dr. Elizabeth R. Constant.

capacity on the skin that any of the substances used in these studies.

In resistant dermatoses, local sulfathiazole therapy has been combined with other types of therapy, including ammoniated mercury ointment, carbol-fuchsin paint, tar ointment, Roentgen ray and ultraviolet therapy, acriflavine and potassium permanganate compresses, and quinolor ointment. There was no indication of incompatibility insofar as the therapeutic results were concerned. We have treated a few patients recently with an ointment containing 5% of sulfathiazole and of sulfanilamide, with no evidence of incompatibility, but also without demonstrating any marked advantage in such a combination.

Conclusions. 1. Sulfathiazole in an oil-in-water emulsion vehicle is effective in the treatment of primary superficial infections of the skin, including staphylococcic or streptococcic impetigo, infectious eczematoid dermatitis, Bockhardt's follicular impetigo, and superficial ecthyma.

2. It is equally effective in acute secondary infection of various preëxistent dermatoses, including particularly pyogenic complications of fungous infections and of dermatitis venenata.

3. Certain criteria for the effective local application of sulfathiazole have been established, and an oil-in-water emulsion vehicle, in which the sulfathiazole is suspended in the external aqueous phase, has apparently met these requirements satisfactorily. A vanishing type stearate base was somewhat less effective than the emulsion type base. Grease bases proved definitely inefficient.

4. Sulfathiazole ointment is useful in chronic deep ulcers of the skin and subcutaneous tissues.

5. In various chronic eczematoid lesions, from which large numbers of staphylococci or streptococci may be recovered, the effects of sulfathiazole ointment are much less striking than in impetigo, but such treatment is occasionally very helpful.

6. There was no evidence of absorption of sulfathiazole in the patients treated, and no local or general toxic reactions.

7. Superficial infections from which streptococci were recovered responded somewhat more rapidly and completely than those yielding staphylococci on culture.

Since this paper was written we have encountered one instance of cutaneous sensitivity to sulfathiazole. In a recent paper, Keeney *et al.** report good results in 60 patients with cutaneous infections treated with 5% sulfathiazole in a base containing equal parts of hydrous wool fat and vanishing cream. In 6 of 8 eczematous infants in which sulfathiazole ointment (amount not stated) was applied to one-half of the body, they found sulfathiazole to be detectable in the blood in concentrations of from 2 to 3.5 mg. per 100 cc. They observed no absorption in adults. In a study of sulfathiazole absorption through the skin of infants and children, now in progress, Jaquette and one of us (D. M. P.) have been unable to demonstrate significant blood levels of sulfathiazole in 10 normal infants weighing from 7½ to 26 pounds following application of 30 gm. of sulfathiazole ointment daily, and in 5 children weighing between 26 and 41 pounds, following application of 60 gm. of ointment (3 gm. sulfathiazole).

* Keeney, E. L., Pembroke, R. H., Chatard, F. E., and Ziegler, J. M.: J. Am. Med. Assn., **117**, 1415, 1941.

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THE CAUSATIVE RELATIONSHIP OF DERMATOPHYTOSIS TO THROMBOANGIITIS OBLITERANS.

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The cause of thromboangiitis obliterans is unknown. Ergot,[10] tobacco,[8] typhus,[7] streptococci,[16] and hormonal disturbances[12] have been proposed as etiologic agents but have not been accepted, as they could not adequately explain the entire syndrome.

In this Peripheral Vascular Clinic, studies in thromboangiitis obliterans revealing a higher incidence of dermatophytosis and a more frequent reaction to trichophytin than in controls were followed in 1939 by the observation of patients with thromboangiitis obliterans in whom acute dermatophytosis immediately preceded attacks of acute migratory phlebitis.

Dermatophytosis is present in many patients with peripheral arterial disease, but it has always been looked upon merely as a complication which must be treated to avoid ulceration and gangrene. As the possibility was kept in mind that dermatophytosis might stand in causative relationship to thromboangiitis obliterans, certain clinical features of thromboangiitis obliterans appeared to be clarified which could not otherwise be explained. Such is the relapsing nature of thromboangiitis obliterans; the limitation of the lesions, in 95% of patients with thromboangiitis obliterans, to the blood-vessels of the legs and arms and the distorted toe nails which

were formerly attributed to ischemia, but which yield fungi on culture.

It should be emphasized that fungi are the one group of pathogenic organisms which are present in the same regions involved by thromboangiitis obliterans and that no investigation has been made as to whether they could or could not produce this vascular disease. As little is known of the relation of fungous diseases to other processes and with clinical observations suggesting that the migratory phlebitis and arteritis in some patients with thromboangiitis obliterans is preceded by a flare-up of dermatophytosis, it was thought worth while to investigate the possibility of this causative relationship. With the exception of a report by Thompson,[17] who also believes in this theory, nothing has been published on this subject.

Dissemination through the blood stream of dermatophytosis, an infection of the skin by fungi such as Trichophyton, Epidermophyton or Monilia, frequently results in the production of secondary lesions which have been called "dermatophytids" or "moniliids." Evidence[13,19] points to sensitization of the skin to the fungi or their products as a cause for these lesions.

Incidence of Dermatophytosis in Thromboangiitis Obliterans. Thirty patients with thromboangiitis obliterans were examined for evidence of dermatophytosis (Fig. 1). Twenty-eight (93%) had dermatophytosis clinically, severe in 20. Of 30 controls, men between the ages of 20 and 50 without thromboangiitis obliterans, 22 (73%) had dermatophytosis clinically, severe in 9. Vesicles, extensive scaling, fissures and involvement of nails were definitely more common among patients with thromboangiitis obliterans than among the controls. On microscopic examination fungi were found in 5 to 12 patients with thromboangiitis obliterans. This is the approximate proportion usually obtained in patients with dermatophytosis.

Skin Tests With Extracts of Fungi. The work of Lewis and Hopper[11] indicates that a positive trichophytin test is indicative of present or past infection by fungi. Skin tests were made with trichophytin* 1:30 in 30 patients with thromboangiitis obliterans. After 48 hours 24 patients (80%) were positive to trichophytin (see Fig. 2). The 2 patients with thromboangiitis obliterans who showed no evidence of dermatophytosis were positive to trichophytin. Of the 30 controls, men between the ages of 20 and 50, without thromboangiitis obliterans, 6 (20%) were positive to trichophytin. Of the 8 controls without clinical evidence of dermatophytosis, none gave a positive reaction to trichophytin. It can be seen from Figure 2 that there were four times as many patients with thromboangiitis obliterans who gave positive skin reactions than those without thromboangiitis obliterans. One patient (J. H.), with thromboangiitis obliterans with slight dermatophytosis, positive for fungus

* Fungus extract made from *Trichophyton interdigitale* (Lederle).

microscopically, upon injection of 0.1 cc. trichophytin into the left forearm, developed erythema and induration of most of the forearm within 48 hours, with a vesicle 2 cm. in diameter at the site of injection.

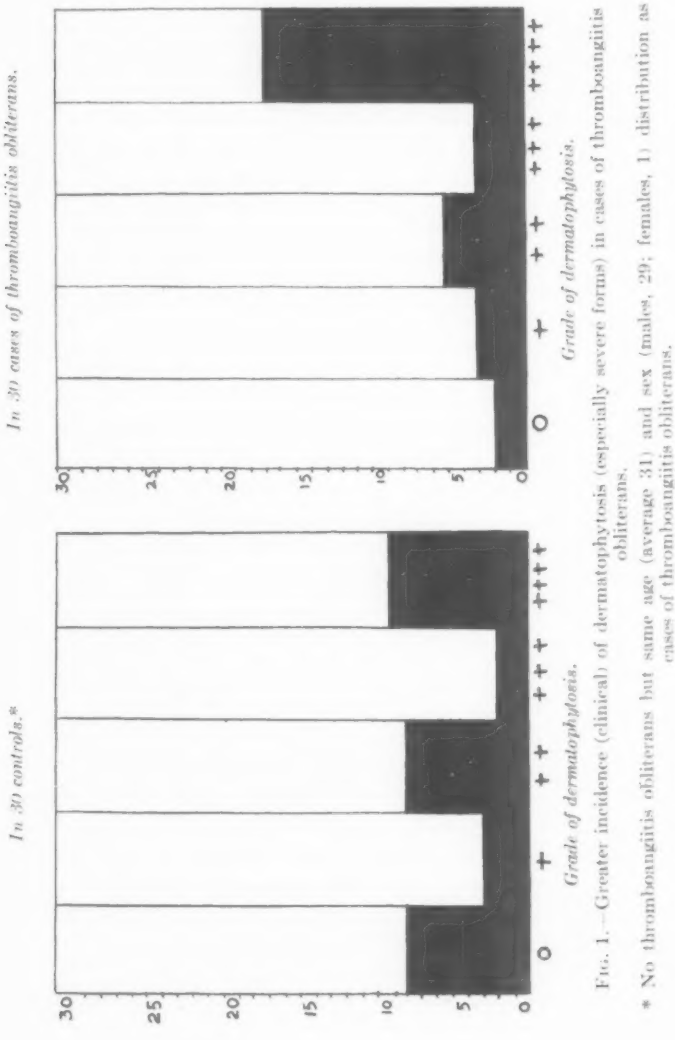


FIG. 1.—Greater incidence (clinical) of dermatophytosis (especially severe forms) in cases of thromboangiitis obliterans.

* No thromboangiitis obliterans but same age (average 31) and sex (males, 29; females, 1) distribution as in cases of thromboangiitis obliterans.

Migratory Phlebitis and Dermatophytosis. Ten of the patients were observed during attacks of migratory phlebitis, characteristic acute lesion of thromboangiitis obliterans. Seven of these had an exacerbation of dermatophytosis preceding the attack of phlebitis,

and on examination all 7 had severe dermatophytosis. The possibility exists that ischemia may have predisposed to dermatophytosis, but there is no evidence to indicate that ischemia favors the development of the fungus disease. Furthermore dermatophytosis and

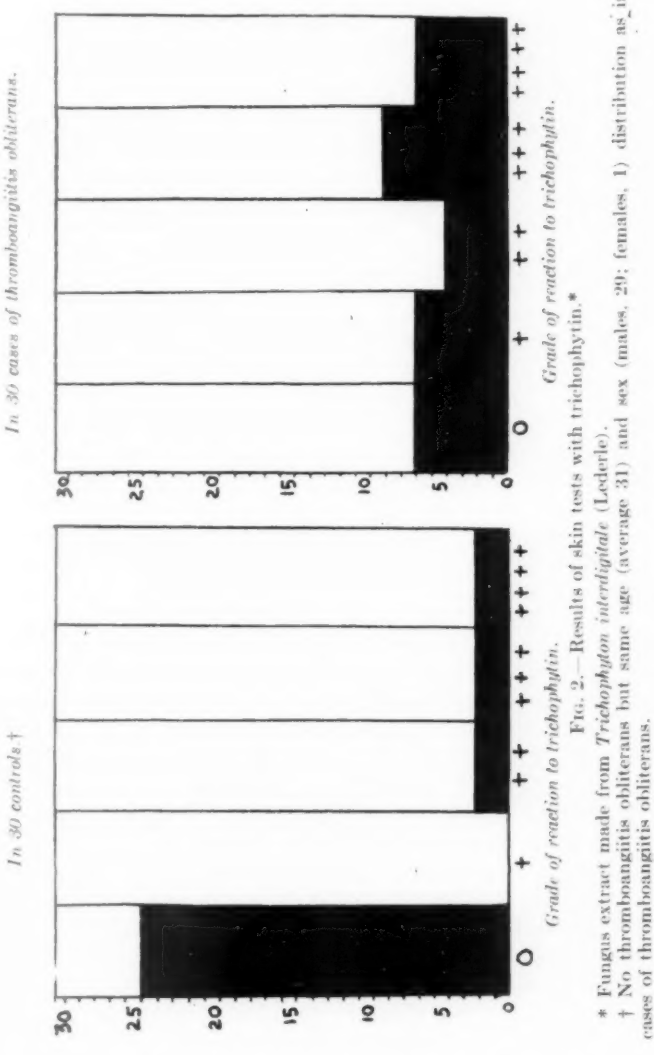


FIG. 2.—Results of skin tests with trichophytin.*

* Fungus extract made from *Trichophyton interdigitale* (Lederle).

† No thromboangiitis obliterans but same age (average 31) and sex (males, 20; females, 1) distribution as in cases of thromboangiitis obliterans.

migratory phlebitis are present in many patients before arterial occlusion and ischemia appear.

The time relationship between dermatophytosis and the appearance of migratory phlebitis is illustrated by the following cases:

J. C., a man of 33, was seen in August, 1939, with a popliteal occlusion and dermatophytosis, the latter being too mild to warrant treatment. In October he returned with a history of severe itching between the toes which had developed 3 weeks previously. Two weeks after the appearance of the itching he noticed red, tender areas along the veins of the right leg. On examination he had a severe, moist type of dermatophytosis between the toes in the involved leg with five areas of acute migratory phlebitis. In the other leg, which had no arterial disease or phlebitis, the dermatophytosis was slight and dry. A skin test with trichophytin which had been done in August was strongly positive.

T. C., a man of 40, was seen first in 1936 with a history of having had dermatophytosis for years. In 1933 he began to have attacks of migratory phlebitis and developed intermittent claudication. He observed that the migratory phlebitis flared up whenever the dermatophytosis became severe. He made wooden shoes so that he could continue taking showers in the plant where he worked without making his dermatophytosis worse. This occurred before he was seen at the University Hospital in 1936. On examination he had occlusion of both popliteal and both ulnar arteries. From 1936 to 1939 he had no exacerbation of his dermatophytosis and no migratory phlebitis. In March, 1939, he developed migratory phlebitis on his hands and in August, 1939, in his right leg and right index finger. Microscopic examination of scrapings from his nails was positive for fungi.

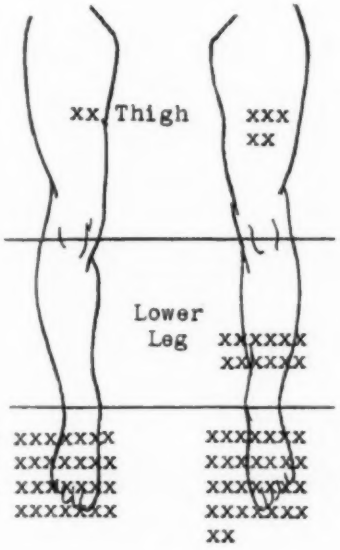
B. T., a man of 56, had a history of thromboangiitis obliterans for the past 15 years. He had no pulses in either femoral artery. On examination in May, 1940, he had a quiet dermatophytosis between the toes and of the nails. In June, 1940, the dermatophytosis became acute and remained so for about a month. In July he came in with acute migratory phlebitis on the dorsum of the foot.

Similarity in the Locations of Inflammatory Lesions of Thromboangiitis Obliterans and Dermatophytosis. Since the fungi which cause dermatophytosis are found in the toes and feet and dermatophytids in the hands, it was thought important to study the location of migratory phlebitis and acute arteritis, the primary lesions of thromboangiitis obliterans (Fig. 3). In 42 patients with thromboangiitis obliterans, migratory phlebitis occurred 30 times in the foot and ankle, 12 in the leg, 5 in the thigh, 11 in the hand and wrist and 4 in the forearm. The frequency with which migratory phlebitis begins in the feet is worthy of comment as other forms of phlebitis occur more frequently in the legs and thighs. That acute arteritis of thromboangiitis obliterans seems to have a similar peripheral distribution at the onset of the disease is shown by the pathologic studies of Buerger.[5] In a typical case the older process is found in the distal portion of the artery, the more recent inflammatory process being found at a higher level. It will be remembered, however, that thromboangiitis obliterans has been reported in parts of the body other than the extremities in about 5% of the patients.[9] Systemic manifestations in dermatophytosis are rare but do occur (Pulvermacher,[15] Bloch,[2] and Bruusgaard[4]).

In thromboangiitis obliterans and dermatophytosis the hands are often involved as well as the feet. In a group of 85 patients with thromboangiitis obliterans 40% with involvement of the feet had

the disease also in the upper extremities. Estimates of the percentage of dermatophytids of the hands in patients with acute dermatophytosis range from 3% by Shelmire[6] to 50% by Loveman.[6]

Age Incidence. In both diseases the great majority of patients fall between the ages of 20 and 50 (White[18] and Williams[20]).



Distribution of dermatophytosis in 30 patients with thromboangiitis obliterans.

Distribution of migratory phlebitis in 42 patients with thromboangiitis obliterans.

FIG. 3.

Relapse. The relapsing nature of thromboangiitis obliterans is well recognized and accounts for the progression of the disease in many patients. More than one attack of migratory phlebitis or arteritis occurs in almost all patients. Dermatophytosis likewise is a stubborn disease and repeated attacks are the rule. Complete cure is difficult and reinfection is common.

The Nails in Thromboangiitis Obliterans. Dystrophy of the nails is mentioned as characteristic of thromboangiitis obliterans (Buerger[5] and Brown, Allen and Mahorner[3]). Examination of a number of patients with thromboangiitis obliterans with deformed, overgrown nails disclosed that these nails do not differ from similar nails seen in patients without vascular disease but with fungous disease of the nails. Cultures of fungi were obtained from the nails of the patient in Figure 4.

Epidemiology of Dermatophytosis and Increase in Incidence of Thromboangiitis Obliterans. In 1921 Anderson[1] reported that between 1914 and 1921 the great majority of severe ringworm infections in immigrants occurred among those coming from the countries of southeastern Europe, the same region from which, according to

Buerger,[5] most of his patients with thromboangiitis obliterans came. Also, many dermatologists believe that following the war there was a great increase in dermatophytosis in this country. This, they attribute to the return of thousands of soldiers with ringworm infection. That this increase of dermatophytosis is more than can be

FIG. 4.—Fungous infection of toe nails in patient, male, age 42, with thromboangiitis obliterans. Culture of scrapings from nails positive for *Trichophyton gypseum*. Skin test with trichophytin positive (see Fig. 5).

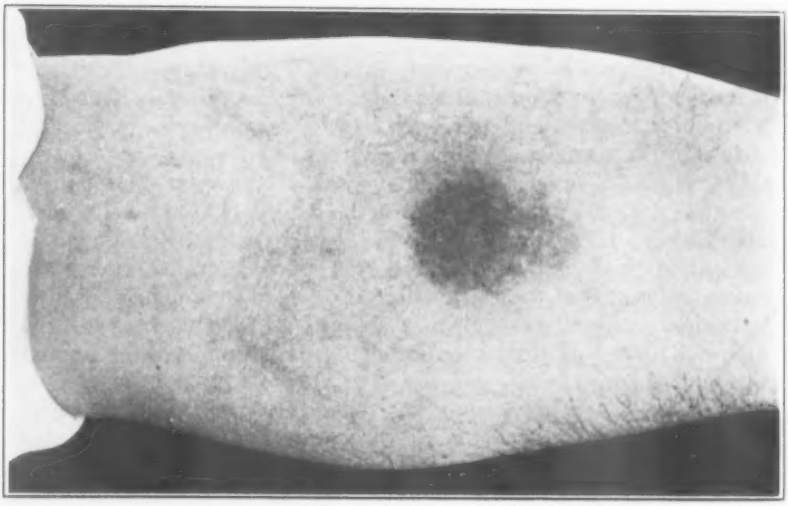


FIG. 5.—Positive skin test in same patient as in Figure 4.

accounted for by increased knowledge of the disease is suggested by White.[18] Concurrently with this increased incidence of dermatophytosis there has apparently been an increase in thromboangiitis obliterans. At this hospital from 1910 to 1919 there were 18 patients with thromboangiitis obliterans, from 1920 to 1929 there were 32 patients and between 1930 and 1939 the number of patients had risen to 114.

Statistics indicate that dermatophytosis is as common among negroes as in the white race. The largest number of cases of thromboangiitis obliterans among negroes in the literature is the 5 reported by Yater,[21] but why this disease is less frequently encountered in that race is not known.

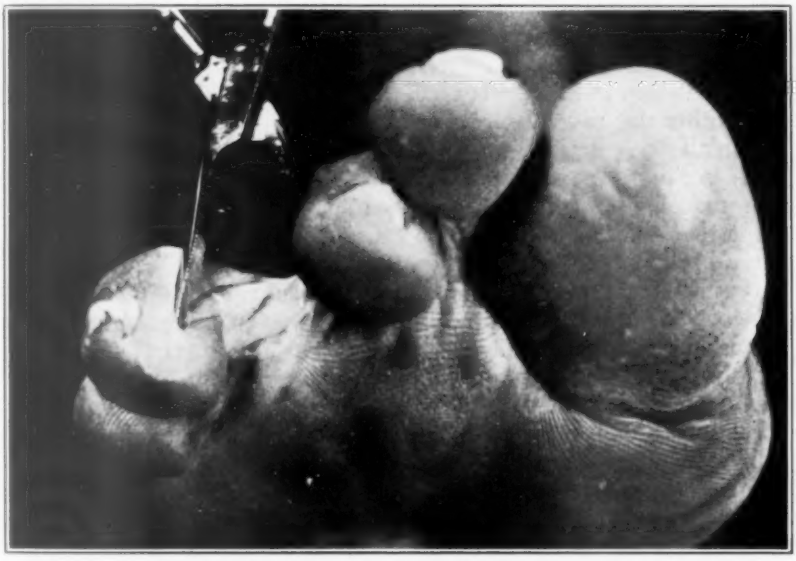


FIG. 6.—Dermatophytosis between toes in patient, male, age 44, with thromboangiitis obliterans. Culture positive for fungus. Positive skin test with trichophytin.

Thromboangiitis obliterans occurs predominantly in men (97%). According to Lewis and Hopper[11] ringworm of the feet and hands is seen more frequently in males than in females. Yet many women have dermatophytosis and if fungi are the cause or precipitating factor in thromboangiitis obliterans, one would expect to find more female patients with the latter disease. A possible explanation of the apparent discrepancy is furnished by Thompson[17] who has stated that certain cases of phlebitis and the ensuing complications may be the counterpart in women of thromboangiitis obliterans in men.

Comment. With the single exception of ergot, which is known to cause vascular disease, the rôle played by fungi in the possible production of thromboangiitis obliterans has never been explored. Ergot has been suggested as a possible cause of thromboangiitis obliterans, but few patients give a history of contact with ergot. On the other hand, most patients with thromboangiitis obliterans harbor the fungi which cause dermatophytosis. These fungi have never been regarded as etiologic or precipitating factors in thromboangiitis obliterans. Phlebitis has been attributed to dermatophytosis in a number of reports (Peck[14] and Pulvermacher[15]). In 1 patient with generalized trichophytid,[15] the phlebitis was thought to have had an allergic basis. In a review of fungous allergy, Peck[14] gives a classification of trichophytids. Among these is a group which he designates "vascular trichophytids" which includes what he terms migrating phlebitis. It is reasonable to believe that in an occasional patient the blood-vessels may act as the "shock tissue" for a reaction to fungi. It is Thompson's[17] belief also that vessels and other tissues may succumb to the products of fungi by means of an allergic reaction.

In seeking a possible relationship between dermatophytosis and thromboangiitis obliterans, one need not necessarily have the concept that the fungi are the sole cause of the vascular disease. It may be that fungi, being concentrated in the blood-vessels of the extremities, act on these vessels in such a manner that other agents such as tobacco or streptococci may bring on an inflammatory reaction. Another possibility is that fungi may precipitate or intensify an inflammatory reaction in blood-vessels previously acted upon by other agents such as tobacco or bacteria. It is not unlikely that more than one agent may cause the inflammatory reaction which has been labeled thromboangiitis obliterans. The combination of the greater frequency of dermatophytosis with the more common use of tobacco in men, with the two factors acting to intensify each other, suggests an explanation for the greater frequency of thromboangiitis obliterans in men.

The prognosis in thromboangiitis obliterans has improved greatly in the past decade. During this period dermatophytosis in these patients has been treated as part of the usual therapeutic regimen because of the fear that the skin disease would open an avenue to infection and gangrene. The improved prognosis is, to be sure, due to many advances in our knowledge of thromboangiitis obliterans and its treatment. However, the incidental treatment of the dermatophytosis may well have been a factor.

Summary. A study has been made of patients with thromboangiitis obliterans with regard to the possibility that the disease may be caused or precipitated by fungi which cause dermatophytosis. While evidence has been presented suggesting an etiologic relationship between the two diseases, it is insufficient, at present, to be

conclusive. Nevertheless, certain clinical features of thromboangiitis obliterans are more readily accounted for on the basis of fungous infection than by other etiologic agents thus far proposed. It therefore seems of primary importance to look for and to treat persistently any evidence of dermatophytosis in the patient with thromboangiitis obliterans.

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VITAMIN A AND ASCORBIC ACID IN PULMONARY TUBERCULOSIS.

DETERMINATION IN PLASMA BY THE PHOTOELECTRIC COLORIMETER.*

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MALNUTRITION is a constitutional factor that affects resistance to tuberculosis.[4-6] Since new objective methods are expanding our knowledge of nutrition rapidly, this field promises much aid in the study of resistance and susceptibility in tuberculosis. The nutritive status is one of many factors which make for resistance or susceptibility. It seems evident, too, that tuberculosis interferes with the normal nutritional balance of the individual. The effect of the disease on the needs for specific nutritive constituents such as minerals, proteins, fats, carbohydrates and vitamins, is being investigated. Of especial interest to us has been the finding of

* Aided by a grant from the John and Mary R. Markle Foundation.

depletion of vitamins A and C in tuberculosis. We have been working with the latter group and have reported in an earlier paper that vitamin A is utilized at an accelerated rate and that this rate of destruction seems to parallel the extent of the tuberculous process.[9] Harris and Harter have also found profound vitamin A deficiency in tuberculosis.[10] Almost universally workers have found a marked deficiency of ascorbic acid, and some have stated that this deficiency parallels the extent of the disease.[1,11] The work reported here attempts to bring new light on the relation of tuberculosis to these two vitamins. It is based on blood tests made on a population of very different character from the Wisconsin population that we studied with the biophotometer.[9]

Selection of Subjects. Most of the subjects were patients attending the chest clinic of this institute for the first time. More than twice as many negroes as white people were tested; persons of Italian descent were the largest nationality group among the white patients. One-half of the negroes and one-third of the white people were dependent upon public assistance for their living. Many of the subjects had no breakfast before reporting at the clinic; and those that did have breakfast had little or no citrus fruit. The effect of breakfast on the ascorbic acid plasma level will be discussed later. Data on the first 100 subjects were accumulated by testing the first 5 new patients each day. Later laboratory facilities were improved so that *all* new patients were tested as they presented themselves. Although this work was done during two periods (April-May, and September-October 1940), we found there was no difference between the first 100 tested and the rest; so all data were pooled. In short, our population group comes from the economically underprivileged areas of Philadelphia where malnutrition in adults as well as children is most apt to occur. Both malnutrition and tuberculosis have a known high incidence in these areas.

Methods. Blood was drawn usually between 10 and 11 A.M. by venipuncture into syringes well oiled with heavy mineral oil to reduce the possibilities of hemolysis and aëration. It was transferred immediately to a specially made 25 cc. ground glass-stoppered centrifuge tube which contained a drop each of 30% potassium oxalate and 5% potassium cyanide for each 5 cc. of blood drawn. The sample was centrifuged immediately; plasma was collected and the 4 cc. portion used for the ascorbic acid test was deproteinized at once. The vitamin A and carotene in the plasma were determined by the Kimble modification of the Dann and Evelyn method[13a] which employs the Carr-Price reaction and the photo-electric colorimeter. Ascorbic acid was determined by reduction of 2,6 dichlorophenolindophenol with metaphosphoric acid plasma filtrates in the Evelyn photo-electric colorimeter. The procedure used was essentially that developed by Kimble and Barbour[13b] at the Wisconsin General Hospital, which combines features of the techniques of Mindlin and Butler[14] and Evelyn, Mallory and Rosen.[5] Erythrocyte sedimentation rate was determined by the method of Cutler.[3]

Results of Vitamin A Tests on Blood Plasma. A. *Staff Members.* A group of 15 staff members composed of technicians, nurses and

physicians was tested as a normal control group to compare with all the patients. The economic status of the staff group was markedly different from the patients and supposedly all were eating complete diets except one who had an aversion to oranges but was eating an otherwise good diet. Both white people and negroes were represented. The individual observations are plotted in Figure 1. The range of the plasma level of vitamin A was classified in 3 groups as follows:

PLASMA LEVELS OF VITAMIN A IN INTERNATIONAL UNITS PER 100 CC.

Sex.	Normal.	Borderline.	Pathologically deficient.
Male	110 and above	85–100	Below 85
Female	95 and above	75–95	Below 75

The lower level of normal shown for the females takes into account the sex difference in the plasma as described by Kimble[13a] and Scalongne.[17] These levels are empiric and undoubtedly will be changed to eliminate the borderline level, when sufficient work has been done to evaluate correctly the various blood levels. These 3 levels were chosen by us after careful consideration of our own data, and after consulting Dr. M. S. Kimble, Wisconsin General Hospital, who has had an experience of over 1000 tests. Some standard is needed to judge the degrees of depletion and to serve as a guide to those attempting to determine optimum nutrition.

In Tables 1 and 2 are listed the per cents of the staff members in each of the 3 plasma levels of vitamin A. None was pathologically deficient and only a few were in the borderline group. Since practically all of the staff members are in the normal group, we believe that our standards for vitamin A have been well chosen. The individual observations are plotted in Figure 1 (see Tables 1 and 2).

TABLE 1.—DISTRIBUTION OF WHITE AND NEGRO PATIENTS ON THE BASIS OF BLOOD LEVELS OF VITAMIN A.

Patient group.	No. of patients.	Normal %.	Borderline, %.	Pathologically deficient, %.
White:				
Non-tuberculous	36	61.1	27.8	11.1
Tuberculous just diagnosed	6	16.6	33.3	50.0
Tuberculous under treatment	13	38.5	23.1	38.5
Negro:				
Non-tuberculous	92	34.8	38.1	27.2
Tuberculous just diagnosed	23	8.7	4.4	87.0
Tuberculous under treatment	15	13.3	13.3	73.3
Staff members (both races)	15	73.3	26.7	0.0

TABLE 2.—DISTRIBUTION OF NON-TUBERCULOUS PATIENTS ON THE BASIS OF BLOOD LEVELS OF VITAMIN A AND RACE AND SEX.

Non-tuberculous patient groups.	No. of patients.	Normal, %.	Borderline, %.	Pathologically deficient, %.
White male	15	53.4	39.9	6.7
White female	21	66.7	19.0	14.3
Negro male	40	25.0	50.0	25.0
Negro female	52	42.3	28.9	28.9
Staff members (both races and sexes)	15	73.3	26.7	0.0

B. *Non-tuberculous Patients.* The results of testing the plasma vitamin A of the non-tuberculous patients as plotted in Figure 1, show a wider range than the staff group. This is to be expected, as these patients have dietary customs which range from the equivalent of the staff members' diets to the starvation diets of those in abject poverty. The fact that most of these patients are very poor probably explains the concentration of the observed readings in the borderline and pathologically deficient levels. Two very high values were observed. One of these may be accounted for by supplementary vitamin intake. The non-tuberculous patients represent

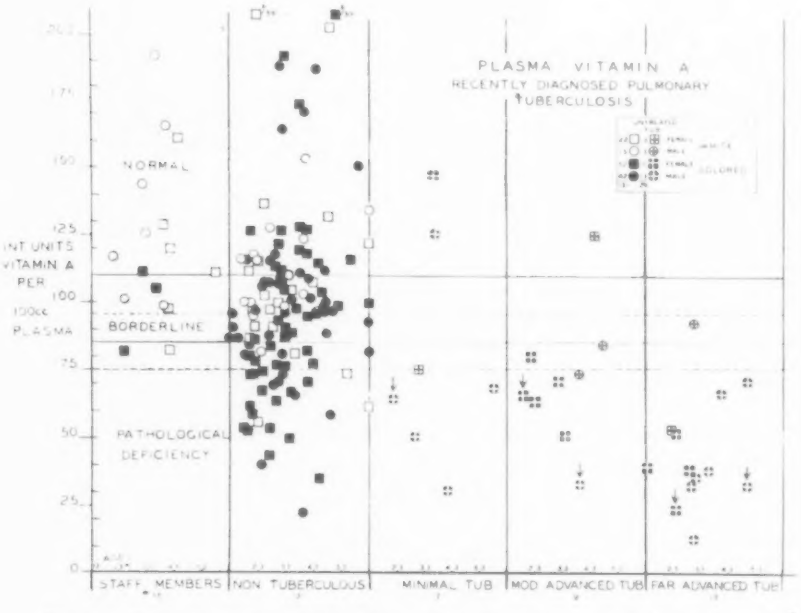

FIG. 1.—Plasma vitamin A observations on staff members, non-tuberculous and tuberculous patients at time of diagnosis. Note that no staff member is pathologically deficient while all but one of the far advanced patients are pathologically deficient. Black arrows indicate deaths.

all the patients coming to the clinic who did not have tuberculosis. Fifteen per cent had common colds and 35% had other upper respiratory infections. A large portion (27%) of the non-tuberculous patients had no known disease and were admitted to the clinic because of recent exposure to an active case of tuberculosis. Table 2 shows that the females of both races had the higher percentage of individuals with normal plasma levels and that they also had the higher percentage with pathologic levels. In Table 2, we believe the race difference in the percentage of patients in the pathologic level is important, as more than twice as many non-

tuberculous negroes (27.2%) were deficient as were white patients (11.1%). Comparing the non-tuberculous groups, 73% of the staff members, 61% of the white non-tuberculous patients, and 35% of the negro non-tuberculous patients, had normal plasma levels of vitamin A. This corresponds with the incidence of tuberculosis in these groups. The patients in the non-tuberculous group are the same in every other respect as the two tuberculous groups, since they are friends, relatives and neighbors of the patients and they constitute the reservoir in which tuberculosis develops. One of the non-tuberculous patients has developed a tuberculous process since the study began. No age difference in the plasma level of vitamin A was observed even when the data were plotted in other ways. If there is an age difference, it is slight and other forces in our population group are more powerful in determining the plasma level of vitamin A.

C. *Tuberculous Patients Just Diagnosed.* During our study, 29 patients entered the chest clinic for the first time and were diagnosed tuberculous and tested by us on the same day. These patients had had no therapy for tuberculosis, as their disease was previously unrecognized. Only 6 of these 29 patients were of the white race. The plasma levels of vitamin A of these patients are plotted in the 3 divisions of the extent of tuberculous involvement in Figure 1. In Table 1, the percentages of these patients falling into the 3 plasma levels reveal a marked drop in the number of individuals with normal plasma levels, *viz.*, to 16.6% for the white and 8.7% for the negro race. This appears to be significant and shows a wide departure from even the low level of nutrition of the non-tuberculous group. There is a corresponding increase in the percentage of patients with pathologically low levels of plasma vitamin A; for the negroes it is 87.0% and for the white race it is 50.0%. This lowered vitamin A level means either: 1, disturbed physiologic processes, which may account for the lowered resistance of our tuberculous patients, especially the negroes; or 2, mere depletion of body stores with no other disturbance. The greater depletion of the negro patients is partly or perhaps wholly due to the smaller amounts present in the body before the disease is contracted.

TABLE 3.—DISTRIBUTION OF TUBERCULOUS PATIENTS ON THE BASIS OF BLOOD LEVELS OF VITAMIN A AND STAGE AND TREATMENT OF TUBERCULOSIS.

Tuberculous patient group.		No. of patients.	Normal, %.	Borderline, %.	Pathologically deficient, %.
Just diagnosed	Minimal	7	28.6	14.3	57.2
	Moderately advanced	9	11.1	22.3	66.6
	Far advanced	13	0.0	0.0	100.0
Under treatment	Minimal	4	50.0	25.0	25.0
	Moderately advanced	8	12.5	25.0	62.5
	Far advanced	16	6.3	31.2	62.5

In Table 3 are listed the percentages of patients in each plasma level for each stage of tuberculosis. The drop in the percentage of

patients in the normal plasma level from 28.6% for the minimal to 0% in the far advanced tuberculous group, and the corresponding rise in the percentage of patients found pathologically deficient in vitamin A from 57.2% to 100%, shows a parallelism between vitamin A deficiency and the extent of tuberculous involvement. This verifies our earlier observation with the biophotometer.[9] This same relationship of relative vitamin A depletion and stage of tuberculosis was observed when the data from the first 100 subjects were analyzed, and when we had tested approximately one-half the number of tuberculous patients. Two patients with far advanced,

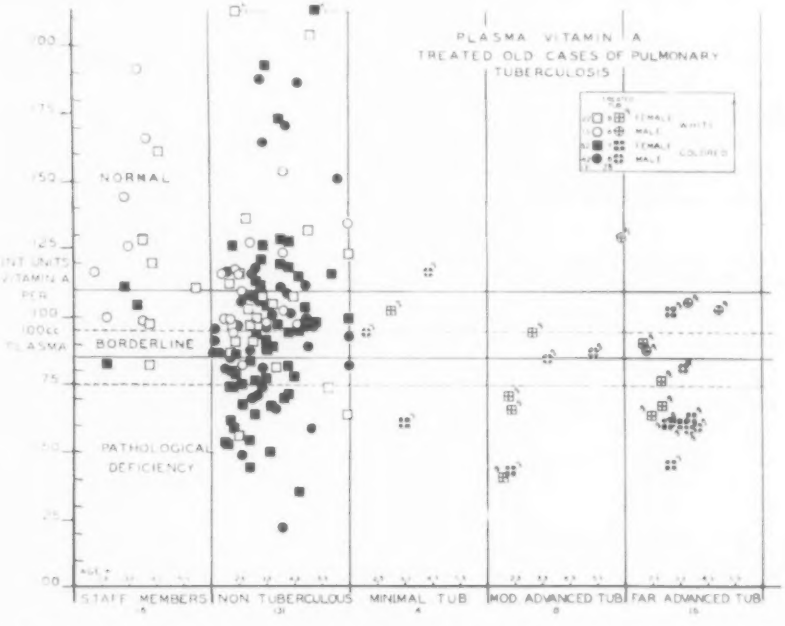

FIG. 2.—Plasma vitamin A observations on staff members, non-tuberculous and tuberculous patients receiving treatment.

2 with moderately advanced and 1 patient with minimal tuberculosis died in the first 3 months after diagnosis. Plasma observations on these 5 patients are marked with arrows in the figures. The two with far advanced tuberculosis that died had the next to the lowest values of plasma vitamin A. The patient with the lowest value in the far advanced group is still alive but critically ill in a hospital. The patient with the lowest value in the moderately advanced group is dead, as is the youngest patient, who had the fourth lowest value in the group. The youngest patient in the minimal group, with the third lowest plasma level of that group, is also dead. All 5 of these tuberculous patients had plasma levels of vitamin A *below*

70 I.U. per 100 cc. and died in 3 months. This strongly suggests that profound hypovitaminosis A in the tuberculous patient makes the prognosis worse.

D. *Patients Receiving Treatment for Tuberculosis.* The plasma level of vitamin A was determined for 28 patients receiving home bed rest. Most of these patients were receiving pneumothorax treatment in addition. No concerted effort with vitamin therapy was carried out; only 4 of these patients were taking cod liver oil, which was not given in sufficient amounts to raise their plasma levels to normal. The observations are plotted in Figure 2; Tables 1 and 3 give the percentages of patients in each of the plasma levels. Of these 28 patients 13 were of the white race. The percentages in the tables show that the patients under treatment have higher plasma levels than the just diagnosed patients. In Table 4 the same relationship of relative depletion that was observed with the just diagnosed group of tuberculous patients is preserved; namely, the percentage of pathologically deficient patients was greatest in the far advanced group and least in the minimal group. The racial handicap still exists for the negroes, as less than one-half as many have normal plasma levels as the white race. There have been no deaths in this group of patients while under observation.

TABLE 4.—DISTRIBUTION OF WHITE AND NEGRO PATIENTS ON THE BASIS OF BLOOD LEVELS OF ASCORBIC ACID ACCORDING TO SEX AND TREATMENT OF TUBERCULOSIS.

Patient group.		No. of patients.	Normal, %.	Borderline, %.	Pathologically deficient, %.
White:					
Non-tuberculous	Male	15	33.3	20.0	46.7
	Female	22	31.8	31.8	36.4
Tuberculous just diagnosed	Male	3	0.0	33.3	66.7
	Female	3	0.0	100.0	0.0
Tuberculous under treatment	Male	5	0.0	0.0	100.0
	Female	9	33.3	44.5	22.2
Negro:					
Non-tuberculous	Male	42	4.8	11.9	83.3
	Female	52	5.8	17.3	77.0
Tuberculous just diagnosed	Male	13	0.0	15.4	84.6
	Female	10	0.0	60.0	40.0
Tuberculous under treatment	Male	8	0.0	37.5	62.5
	Female	8	25.0	62.5	12.5

E. *Plasma Vitamin A and Weight.* In Figure 3 are plotted all the observations on plasma vitamin A against per cent of standard weight for each patient. All but 5 of the tuberculous cases are under 100% standard weight and 2 of these 5 are under treatment. Oddly enough the tuberculous individual with the highest per cent of standard weight (113%) was a "just diagnosed" individual who died within 3 months of tuberculosis. Only 1 non-tuberculous white woman was overweight, with more than 120% of standard, but there were 19 negroes overweight more than this figure. In Figure 3 we see that more overweight people had normal plasma

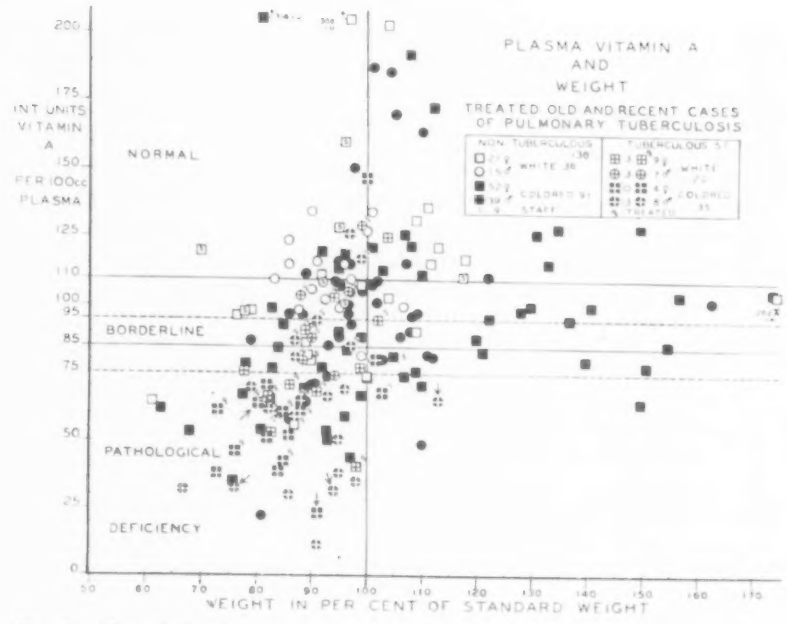

FIG. 3.—The relation between plasma vitamin A and per cent standard weight for all subjects.

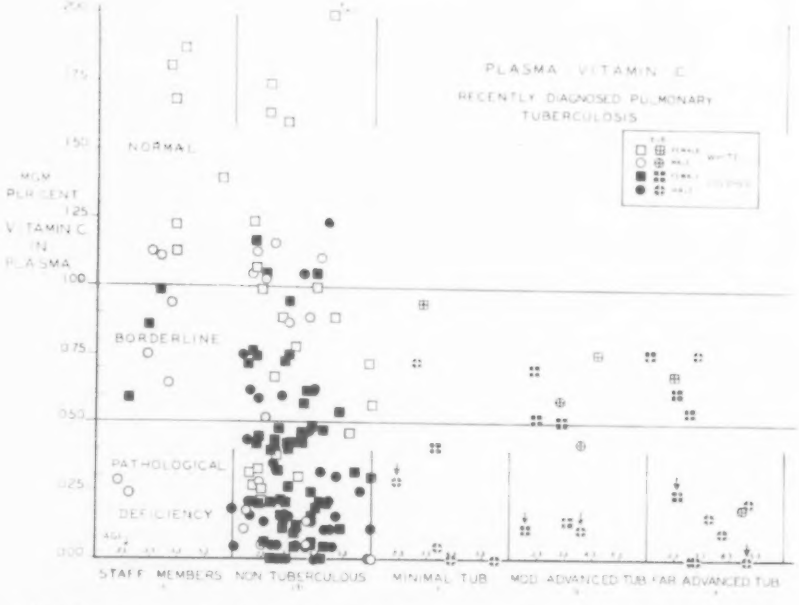

FIG. 4.—Plasma vitamin C observations on staff members, non-tuberculous and tuberculous patients at time of diagnosis.

levels of vitamin A than did those who were underweight, and 9 overweight patients had pathologically low levels of plasma vitamin A. Obviously overweight individuals are not necessarily "well nourished" with respect to vitamin A.

F. *Results of Carotene Tests on the Plasma of All Patients.* The plasma carotene levels of the various groups of patients will be reported in a separate paper together with the results of other work with carotene.

Results of Ascorbic Acid Tests on Plasma. A. *All Patient Groups.* In Figure 4 are plotted the observations on the plasma level of ascorbic acid for each of the subject groups. The plasma range of ascorbic acid levels is divided into 3 groups; normal, borderline and pathologically deficient. Some workers would place the limit of the normal group at 0.7 mg. per 100 cc. of plasma,[8,12] but we have found no reason to change the standard of 1 mg. per 100 cc. We realize that this standard has been set empirically and that the true limit of normal is yet to be determined. Almost all workers agree that the prescorbutic zone or pathologically deficient zone is below 0.5 mg. per 100 cc. Using these standards, only 50% of the staff members had plasma levels in the normal range, 37.5% had borderline levels, and 12.5% were pathologically deficient.

The staff members in the borderline and pathologically deficient groups (except one) were used in another experiment in which they were given orange juice* equivalent to 250 mg. of ascorbic acid daily. The staff member with the lowest ascorbic level was known to have an aversion to oranges. When tested 3 weeks later, all had normal plasma levels. This would indicate that their dietary intake of ascorbic acid had been too low and that our standard levels are well chosen.

The non-tuberculous patients have a wider range of plasma levels of ascorbic acid than the staff members and there is a gross difference in the distribution of observed levels as shown in Figure 4. A little over 30% of the white patients (both sexes) had normal plasma levels, as compared with 5 to 6% for the negroes (Table 4). Forty-seven per cent of the white males and 36% of the white females had pathologically low levels, while 83% of the male negroes and 77% of the female negroes had pathologic levels.

The just diagnosed tuberculous subjects had even lower levels of ascorbic acid. *None* had a normal plasma level (Fig. 4, Table 4); however, in this markedly deficient group the females of both races show levels higher than the males. In Table 6 are listed the number, instead of the usual per cent of individuals, in the 3 plasma levels, since the number of subjects became small when divided on a sex basis. There is a rough parallelism between the extent of the disease and the number of individuals with pathologically low

* Courtesy of California Fruit Growers Exchange.

levels of ascorbic acid. It is more easily seen in the figures for the males than in the figures for the females.

In Figure 5 are plotted the same observations for the staff members and non-tuberculous patients as in Figure 4, but the other observations are for the tuberculous patients under treatment. In Table 4, *none* of the males of either race is shown to have a normal plasma level. Again, as with vitamin A, the treated tuberculous patients show better plasma levels than the just diagnosed tuberculous patients; in fact, the range of plasma levels is indistinguishable from the non-tuberculous group. The change for the females is

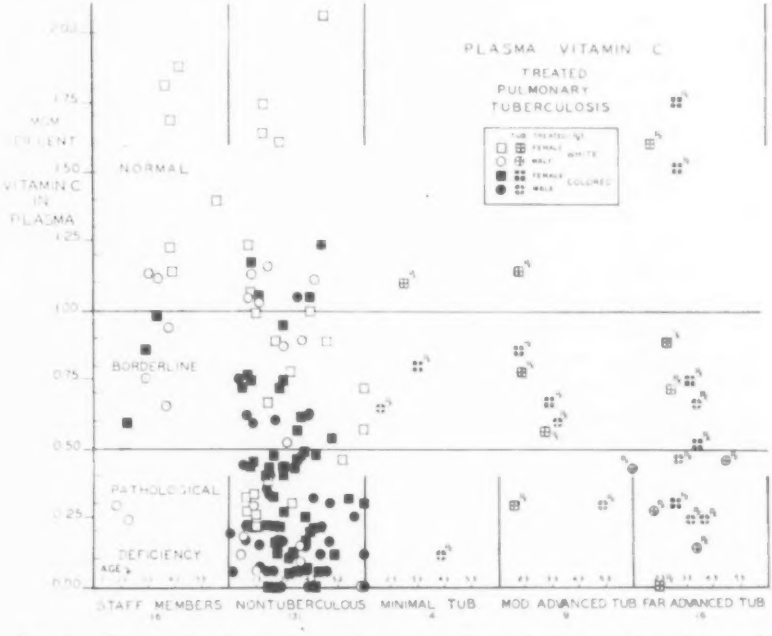

FIG. 5.—Plasma vitamin C observations on staff members, non-tuberculous and tuberculous patients receiving treatment.

particularly evident in the number and per cent that are now in the normal plasma level (Table 4). In Table 5 are listed the number of patients in each plasma level by stages of tuberculosis, and again we see the rough parallelism between depletion and extent of tuberculous involvement.

B. *Plasma Ascorbic Acid and Weight.* In Figure 6 are plotted the individual observations of plasma ascorbic acid against per cent of standard weight for each patient. Eleven of the 28 subjects with a normal level of ascorbic acid were underweight, weighing less than 90% of standard weight. Of the 204 subjects, 37 were overweight, weighing more than 110% of standard weight; 26 of these 37 over-

weight subjects had plasma ascorbic acid values in the pathologically deficient level, and 18 of the 26 were female non-tuberculous negroes. This observation that *26* of the 37 overweight subjects had pathologically low plasma levels of ascorbic acid shows that overweight patients are not always "well nourished" in regard to ascorbic acid.

TABLE 5.—TUBERCULOUS PATIENTS GROUPED ON THE BASIS OF BLOOD LEVELS OF ASCORBIC ACID AND SEX, STAGE AND TREATMENT OF TUBERCULOSIS.

Patient groups.	Males, No. of patients.			Females, No. of patients.		
	Normal.	Borderline.	Pathologically deficient.	Normal.	Borderline.	Pathologically deficient.
Just diagnosed:						
Minimal	0	1	4	0	1	1
Moderately advanced	0	1	2	0	4	2
Far advanced	0	1	7	0	4	1
Under treatment:						
Minimal	0	1	1	1	1	0
Moderately advanced	0	1	2	1	4	1
Far advanced	0	1	6	3	4	2

TABLE 6.—PERCENTAGE OF PATIENTS IN EACH PATIENT GROUP WITH NORMAL PLASMA LEVELS AND PATHOLOGIC PLASMA LEVELS OF VITAMIN A, VITAMIN C, AND BOTH VITAMINS A AND C.

Patient group.			Total No.	Plasma level of—					
				Vitamin A.		Vitamin C.		Both vitamins A and C.	
				Normal, %.	Pathological, %.	Normal, %.	Pathological, %.	Normal, %.	Pathological, %.
Staff members			15	73.3	0.0	50.0	12.5	47.0	0.0
Non-tuberculous	White	Male	15	53.4	6.7	33.3	46.7	13.0	0.0
		Female	21	66.7	14.3	31.8	36.4	23.8	9.5
	Negro	Male	40	25.0	25.0	4.8	83.3	0.0	22.2
		Female	52	42.3	28.9	5.8	77.0	0.0	19.2
Tuberculous just diagnosed	White	Male	3	0.0	66.7	0.0	66.7	0.0	66.7
		Female	3	33.3	33.3	0.0	0.0	0.0	0.0
	Negro	Male	13	7.6	92.4	0.0	84.6	0.0	77.0
		Female	10	10.0	80.0	0.0	40.0	0.0	30.0
Tuberculous under treatment	White	Male	4	75.0	0.0	0.0	100.0	0.0	25.0
		Female	9	22.3	55.5	33.3	22.2	11.1	22.2
	Negro	Male	8	12.5	62.5	0.0	62.5	0.0	37.5
		Female	7	14.3	85.6	25.0	12.5*	14.3	14.3

* Eight patients.

Percentages in this table do not add to 100% because patients with borderline plasma levels of either or both vitamins are not included.

Plasma Ascorbic Acid Versus Plasma Vitamin A. In order to study the distribution of patients with multiple vitamin deficiencies, all observations on the plasma vitamin levels are plotted against each other in Figure 7. We note that the upper right hand corner is the region where observations are plotted on patients that had a normal plasma level of both vitamins A and C. In the lower left-hand

corner are plotted the observations on patients with pathologically deficient plasma levels of both vitamins A and C. All 5 of the patients that died within 3 months after being diagnosed were in the latter group (Fig. 7).

In Table 6 are listed the number and per cent of the patients in each group that have normal plasma levels of both vitamins and pathologically low levels of both vitamins. The per cent of patients in each group with a normal plasma level of only one vitamin is also given, and, conversely, the per cent with a pathologically deficient plasma level of only one vitamin is given. The 16 patients that

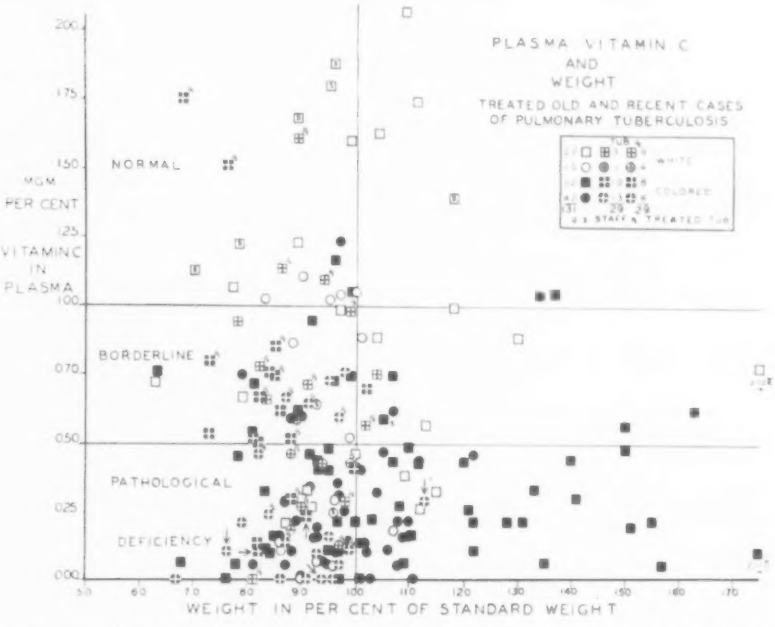

FIG. 6.—The relation between plasma vitamin C and per cent standard weight for all subjects.

had a normal plasma level of both vitamins were largely made up of staff members and white females (Table 6). The 43 patients with plasma levels revealing pathologic deficiencies of both vitamins were largely negroes, 14 females and 20 males. This shows a marked difference in the nutrition of the white and negro races in our population group.

Plasma Ascorbic Acid and Erythrocyte Sedimentation Rate. Since many far advanced cases of tuberculosis show a profound lack of ascorbic acid and have a high erythrocyte sedimentation rate, one might suspect a cause and effect relationship. In Figure 8 are plotted the plasma ascorbic acid observations against the erythro-

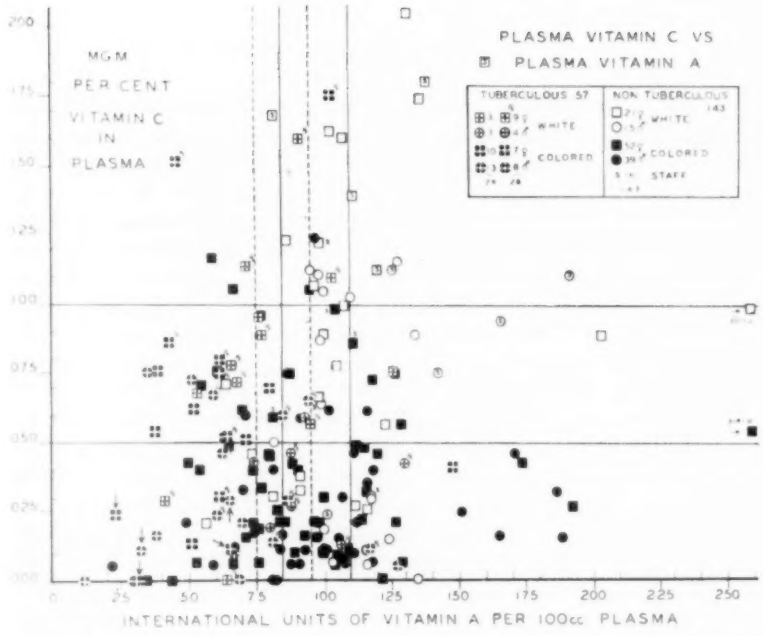

FIG. 7.—Plasma vitamin C plotted against vitamin A. The vertical and horizontal lines demarcate the plasma levels for each vitamin as before. Note that those individuals with normal plasma levels of both vitamins, in the upper right corner, are largely staff members and non-tuberculous white females. Those individuals with low levels aggregated in the lower left include the bulk of the tuberculous patients. Note especially that the 5 tuberculous patients who died had pathologically low levels of both vitamins.

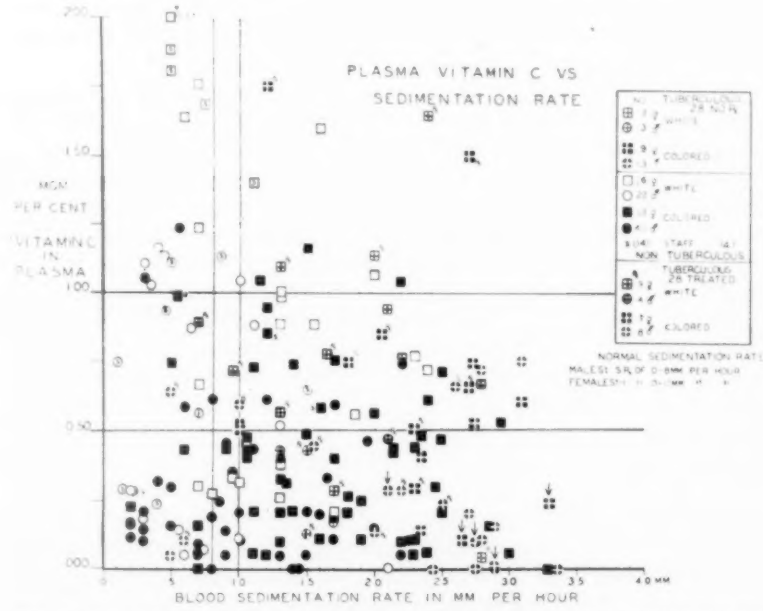

FIG. 8.—The relation between plasma vitamin C and blood sedimentation rate for all subjects.

cyte sedimentation rate for each patient. A few patients with high normal plasma levels of ascorbic acid also had normal erythrocyte sedimentation rates, and also a few with normal ascorbic acid plasma levels had rapid erythrocyte sedimentation rates. It is true that the largest group of patients had an abnormally high erythrocyte sedimentation rate and a pathologically low plasma level of ascorbic acid, but 32 patients (16%) had pathologically low plasma levels of ascorbic acid and had a normal erythrocyte sedimentation rate. This, we believe, shows that the ascorbic acid deficiency and a rapid erythrocyte sedimentation rate may coëxist but they *may also exist independently.*

The Effect of Breakfast on the Plasma Levels of Vitamins A and C. Five male negroes were studied to determine the effect of breakfast on the plasma levels of both vitamins A and C. Three of the 5 had come to the clinic without breakfast and the other 2 had breakfast without fruit. All 5 were bled on entrance and were immediately given 100 mg. of crystalline ascorbic acid* in 2 ounces of distilled water by mouth. This was the equivalent of a good breakfast of fruit containing ascorbic acid. One and one-half hours later they were bled a second time. The curve of the plasma level of ascorbic acid reaches a peak at about 3 hours after eating according to our data, which agree with those of Farmer and Abt.[7] At one and a half hours the rise is about one-half as great as it is at the peak. All 5 patients had pathologically low levels of plasma ascorbic acid and failed to show any appreciable rise in their plasma level in one and a half hours. The rise for each patient was 0.04, 0.01, 0.08, 0.02 and 0.01 mg. per 100 cc. from 0.13, 0.17, 0.26, 0.00 and 0.06 mg. per 100 cc. plasma ascorbic acid, respectively.

These 5 patients were given no additional vitamin A and the 2 that had breakfast, with some vitamin A in the butter and cream, were compared to the 3 without breakfasts. One of the 2 with breakfast showed no rise in his plasma vitamin A 3 hours after eating. The other patient with breakfast was not bled at the time of eating but was bled $3\frac{3}{4}$ and $5\frac{1}{4}$ hours after eating. His last bleeding showed 63.5 I. U. per 100 cc. plasma while the first one showed only 41.8 I.U. per 100 cc. plasma. This boost, however, was not sufficient to raise his blood level from the pathologically deficient group. The 3 patients without breakfast changed their plasma levels very little in $1\frac{1}{2}$ hours. The changes in vitamin A were from 42.6, 57.3 and 108.0 to 45.4, 63.5 and 110.2 I.U. per 100 cc. plasma respectively. The slight rise in each case is insignificant, although the last case was boosted into the normal plasma group.

A healthy subject (H.G.) was given a breakfast including orange juice to the equivalent of 100 mg. of ascorbic acid and was bled at $1\frac{1}{2}$ hour intervals. One and a half hours after eating, his plasma level had risen from 1.34 to 1.46%. Three hours after eating it

* Courtesy of Merck & Co.

was 1.66 mg. per 100 cc. and at 4½ hours it had dropped to 1.45 mg. per 100 cc. In the first 1½ hours the rise was 0.12 mg. per 100 cc. which was more than that of the best of the 5 patients, who had a rise of 0.08 mg. per 100 cc. The plasma vitamin A on the normal subject who ate 750 I.U. in milk and butter for breakfast was very stable. One and a half hours after eating it had changed from 133 to 129 I.U. per 100 cc. plasma, at 3 hours it was 139 I.U. and at 4½ hours 136 I.U. of vitamin A per 100 cc. plasma.

All the variations with breakfast were greater with vitamin C, but were not sufficient to alter seriously our distribution of patients in the 3 plasma levels. More than one-half of our new patients come without breakfast and those that do have breakfast rarely have as much as we gave our 5 test subjects. As we have demonstrated, 100 mg. of ascorbic acid does raise the plasma level more in a subject that has a normal level than in one whose tissues are unsaturated. Any errors in placing the patients in the proper plasma levels would all be in one direction. The tendency would be to boost a few patients that belong in the next lower level into the normal and borderline groups. This boosting is insignificant, as it would also tend to make our figures show less malnutrition than actually exists, while, as they are, they show profound malnutrition of our population in respect to ascorbic acid and a less but severe malnutrition in respect to vitamin A.

We are aware that alcoholic beverages may free stored vitamin A and thus falsely high plasma levels may be observed. Those of our patients, however, that drank alcoholic beverages apparently had exhausted their body stores by previous indulgence, for their plasma levels were lower in most instances than the group average. One tuberculous patient was thought to have cirrhosis of the liver and his plasma level was very low.

Comment. The vitamin A level in the blood stream is fairly stable and not affected by meals with the usual amounts of vitamin A and carotene consumed. This means that blood samples may be taken at any time without any great error in the assessment of nutritional status. If the peaks of fat absorption are avoided (3 to 6 hours after eating) there is still less chance for error. Ascorbic acid plasma values show a rise following each meal, which is greatest at about 3 hours. Ascorbic acid, being water soluble, is stored much less than vitamin A, as has been shown by Crandon and Lund.[2] A fasting plasma level would be preferable whenever possible.

The sex difference in plasma level of vitamin A has been observed before by Kimble[13a] and Scalongne,[17] and our data would substantiate their observations and would support a similar claim for ascorbic acid. With vitamin A, the females have a lower average plasma level, while with ascorbic acid it is just the reverse. This is particularly noticeable in the negro females in each patient group; the number deficient in ascorbic acid was always less than among

the negro males. Our figures are too small to prove such a contention definitely, but the data point out the possibility of a sex difference.

McConkey[15] has shown that vitamin A and ascorbic acid play a rôle in the resistance to tuberculosis, as the incidence of intestinal tuberculosis was less in the treated group. The extensive work of Sweany *et al.*[18] also shows that ascorbic acid has therapeutic value in tuberculosis. Osborn and Gear[16] have pointed out that resistance to tuberculosis parallels the animal's ability to produce its own ascorbic acid. Our data, however, would single out vitamin A as the vitamin more closely allied with resistance. The fact that the young female negro patient is more susceptible to tuberculosis and more apt to be deficient in vitamin A and more apt to have a better ascorbic acid level than the negro male, supports this claim. We do not mean by this that vitamin A nutrition should be considered without respect to the other nutritive constituents. Undoubtedly, the "sparing and sharing" relationship between the vitamins necessitates optimum nutritive levels of all the constituents to obtain maximum resistance to infections. What is optimum for resistance to infection may be different from the optimum nutritive level for growth and probably distinctly different from the minimum nutritive level necessary to meet our accepted growth standards. Our vitamin-deficient overweight patients warn against the folly of considering overweight patients necessarily "well nourished." Wetzel's[19] determination of "nutritional grade" takes into consideration only those nutritive constituents that affect a minimum standard of growth and does not consider the optimum nutrition which is best for all body functions including resistance to infections and growth. We can learn the importance of malnutrition in resistance to infections only by studying tuberculous patients' requirements of specific nutritional substances. We find that tuberculosis alters the normal nutritive balance of at least two substances: heavy demands are made on the body for vitamins A and C and these demands are in proportion to the extent of the disease.

Conclusions. 1. The level of vitamin A in the plasma of tuberculous patients was lowered in proportion to the extent of tuberculous involvement.

2. The level of ascorbic acid in the plasma was lowered with tuberculous involvement and reached prescorbutic levels with advanced tuberculosis.

3. Pathologic deficiency of both vitamins A and C appears to make the prognosis worse in tuberculosis.

4. Non-tuberculous patients in the dispensary population studied were malnourished in both vitamins A and C.

5. Patients receiving bed rest and pneumothorax treatment had more vitamins A and C in their plasma than patients just diagnosed.

6. Females (white and negro) had less vitamin A and more ascorbic acid than males.

7. Ascorbic acid deficiency and rapid erythrocyte sedimentation may coëxist but they also exist independently.

8. No age difference in the amount of vitamin A in the plasma was observed.

9. Many overweight patients were found pathologically deficient in vitamins A and C.

10. Negroes were found to be more malnourished in both vitamines than were the white subjects.

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EXPERIMENTAL HYPOPROTHROMBINEMIA.

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The study of a group of hypoprothrombinemic patients that did not respond to vitamin K or its potent synthetic substitutes has led us to review the experimental knowledge of hypoprothrombinemia in the hope of defining the vitamin K-fast group of patients on an etiologic basis and in the hope of finding an effective therapeutic regimen.

A reduction in the concentration of the plasma prothrombin has been effected experimentally by at least 10 different means (Table 1). This laboratory has had first-hand experience with 6 of them.

I. *K Avitaminosis.* The first method may be described as pure dietary K avitaminosis. It was used accidentally by Dam,[8a,b] by McFarlane, Graham and Richardson,[19] by Holst and Halbrook[15] and by Almquist and Stokstad.[1] It is effective on newly hatched chicks, ducklings and goslings. It was effective in a rabbit (Dam and Glavind[9a] and in 12 of 76 rats (Greaves[11]). Early attempts to use the method in man failed (Dam and Glavind[9b]) but recently Kark and Lozner[17] reported success in 4 patients. Hemorrhagic disease of the newborn is regarded by Quick[22c] as a simple K avitaminosis.

TABLE 1.—EXPERIMENTAL METHODS OF PRODUCING HYPOPROTHROMBINEMIA.

Method.	Species in which it is known to be effective.	Time required in first species mentioned.	Response to vitamin K.	
			Without bile salts.	With bile salts.
I. Dietary K avitaminosis	Chicks, rats, rabbits and man	4 to 14 days	Excellent	Excellent
II. Biliary fistula	Rats, dogs and man	21 to 28 days	Slight	Excellent
III. Common duct obstruction	Rats	21 to 28 days	Slight	Excellent
IV. Phosphorus poisoning	Dogs	12 hrs.	Poor	Poor
V. Chloroform anesthesia	Dogs	12 hrs.	Poor	Poor
VI. Carbon tetrachloride poisoning	Dogs and rats	12 hrs.	Poor	Poor
VII. Sweet clover disease	Cattle and rabbits	...	Good	
VIII. Hepatectomy	Dogs and cats	1 hr.	Poor	Poor
IX. Liver massage	Dog	24 hrs.		
X. Changes in the circulation of the liver	Dog	2 to 4 wks.		

Times are approximate and subject in most instances to individual variation. A poor response indicates that *little* or *no* improvement results from vitamin K therapy.

II. *Sweet Clover Disease.* Sweet clover disease of cattle is associated with a hemorrhagic tendency which Roderick[24] showed to be due to a hypoprothrombinemia. Quick[22a] has made a study with "spoiled" sweet clover hay on rabbits and believes that the toxic material destroys prothrombin directly and that the effect is not due to liver damage. The difficulty of extending observations on sweet clover disease lay in the fact that the toxic clover hay was not always available.

III. *Biliary Fistula.* A third method consists in diverting the bile from the intestinal tract by a complete internal or external biliary fistula. An internal fistula established by anastomosis of the gall bladder to the pelvis of the right kidney in the manner described by Kapsinow, Engle and Harvey[16] was employed by Hawkins and Whipple.[14] A hemorrhagic diathesis gradually developed in their animals over a period of from 2 to 4 months. Later Hawkins and Brinkhous[13] showed that the hemorrhagic tendency produced by this method was due to a decline in the plasma prothrombin concentration. Several other investigators have since used this method for producing hypoprothrombinemia. It is often very slow. Two animals in this laboratory failed to show any striking change in prothrombin concentration 15 weeks after the establishment of a complete internal biliary fistula, of the type just described.

Greaves and Schmidt[12] obtained a prothrombin deficiency in rats in a somewhat shorter time by transplantation of the common duct into the distal transverse colon. They demonstrated that the condition could be quickly corrected by the administration of vitamin K. Small doses were effective if bile salts were given, whereas very large amounts were necessary if bile salts were not administered. They, therefore, concluded that bile salts were required for the absorption of the vitamin. This was confirmed in the dog by Smith, Warner, Brinkhous, and Seegers[26] and has formed the basis for the treatment of hypoprothrombinemia in patients with obstructive jaundice or biliary fistula.

IV. *Common Duct Obstruction.* Simple obstruction of the common bile duct has not been a satisfactory method of producing hypoprothrombinemia in the dog. One of our dogs survived common duct obstruction for 13 weeks without developing any marked prothrombin deficiency. In the rat, however, Greaves and Schmidt[12] were able to produce hypoprothrombinemia by this method.

V. *Chloroform Anesthesia.* VI. *Carbon Tetrachloride Poisoning.* VII. *Phosphorus Poisoning.* These three means of reducing the prothrombin concentration in animals are so similar that they may be considered together. The administration of chloroform anesthesia and of phosphorus given hypodermically were first studied by Smith, Warner and Brinkhous[25a] who showed that a severe drop in the plasma prothrombin concentration occurred after 90 minutes of chloroform anesthesia. They observed further that the drop started in 12 hours and reached a maximum within 48 hours. If the animals survived, the prothrombin level then gradually rose to normal over a period of 5 or 6 more days. These authors called attention to a difference between the effect of chloroform on the prothrombin concentration and its effect on the fibrinogen concentration of the plasma. They found that the prothrombin level was affected more easily than was the fibrinogen concentration, that it declined more quickly and that it returned to normal more slowly than was the case with fibrinogen. Quick[22b] reported that carbon tetrachloride exerts an effect on prothrombin comparable to that exerted by chloroform. These experiments were quickly interpreted as evidence that the liver produced prothrombin. Later work tended to confirm this view but it is unsafe to conclude that effects observed following the administration of hepatotoxic agents are mediated solely by damage to the liver since simultaneous injury to other body tissues cannot always be excluded. The hepatic injury which follows the use of such substances results in a reduction of bile salt synthesis and consequently may interfere with the absorption of vitamin K from the intestine.

VIII. *Partial Hepatectomy and Total Hepatectomy.* Partial hepatectomy was carried out in rats by Warner.[28] If 60% to 75% of the liver tissue of these animals is removed the prothrombin level declines. Warner found that the average decline of groups of rats

corresponded somewhat to the extent of the operation but that there were rather wide individual variations. Complete hepatectomy is followed by a precipitous fall in the plasma prothrombin concentration in dogs as was shown by Andrus, Lord and Moore[3] and by Warren and Rhoads.[30]

IX. *Liver Massage.* Moderate declines in prothrombin concentration have been produced by simple massage of the liver through laparotomy wounds (Lord[18]).

X. *Disturbances in the Circulation of the Liver.* Finally, disturbances in the circulation of the liver may, at times, result in pronounced changes in the prothrombin level, as in some of the dogs hepatectomized by us according to the method of Mann as described by Markowitz.[20] The first stage of hepatectomy by this method consists in anastomosing the portal vein to the inferior vena cava and ligating the latter cephalad from the anastomosis. After allowing a period of 4 weeks for the establishment of adequate collateral circulation the portal vein is ligated cephalad from the anastomosis. The actual removal of the liver is carried out 3 weeks after the second stage. Two of the animals in which hepatectomy was done by this method had plasma prothrombin concentrations respectively of only 40% and 45% of normal at the beginning of the third stage.

The Basic Mechanisms by Which Hypoprothrombinemia Develops. Before discussing some of these methods in greater detail the mechanisms by which hypoprothrombinemia is produced experimentally may be rapidly reviewed. The first is simple K avitaminosis, the second a lack of bile salts in the intestinal tract resulting in a conditional K deficiency, due to the failure of absorption of the vitamin, the third is the unexplained action of a toxin on the plasma prothrombin and the fourth is deficient prothrombin production due to damage to the liver. So far, hypoprothrombinemia due to an inability of the wall of the small intestine to absorb vitamin K has not been reported in animals, although it has been postulated as the cause of hypoprothrombinemia in patients with sprue and celiac disease (Butt, Snell and Osterberg[7]). The natural occurrence of human hypoprothrombinemia due to the direct action of toxic substances on prothrombin has not been reported.* There remain, therefore, three mechanisms of clinical significance on which experimental data are available, namely, lack of vitamin K, lack of bile salt in the intestine for its absorption, and liver damage.

The amount of time required for the production of hypoprothrombinemia by any given method in the experimental animal is to some extent indicative of the mechanism by which it is produced. The dietary methods usually require 4 days to 3 weeks. The bile de-

* The active principle of spoiled clover hay (3-3′ methylenebis 4-hydroxycoumarin) isolated in the department of Dr. K. P. Link has recently been employed by H. R. Butt, E. V. Allen and J. L. Bollman (Proc. Staff Meet. Mayo Clin., **16**, 388, 1941) and in patients by J. B. Bingham, O. O. Meyer and F. J. Pohle (this journal, **202**, 563, 1941).

privation methods usually require 4 weeks to 4 months. The chemical methods of liver injury require 12 to 48 hours and following total hepatectomy the prothrombin level begins to fall almost at once. One cannot, of course, conclude that the same time intervals will be operative in man as in the experimental animals, but a sudden drop in plasma prothrombin concentration in a patient is suggestive evidence of the development of hepatic damage.

Damage to the hepatic parenchyma nearly always results in a diminished bile salt content of the bile so that two mechanisms may operate together when such damage is present. In cases in which hypoprothrombinemia develops rapidly it would seem probable that the hepatic injury *per se* plays the dominant rôle. That this is actually the case following chloroform anesthesia in dogs is shown by the following experiments:

Experiments to Determine the Mechanism of the Development of Hypoprothrombinemia Produced by Chloroform and Carbon Tetrachloride. Stock dogs, which had been kept on a diet of table scraps, were operated upon under ether anesthesia. The common duct was divided and both ends intubated with small rubber catheters which led through stab wounds to the surface of the abdominal wall where they could either be connected if the normal flow of bile was to be restored, or separated to sidetrack the bile and to facilitate the collection of specimens. The cystic duct in these animals was ligated with silk. After the animals had recovered from the operation and with the catheters connected so that bile flowed into the duodenum, chloroform anesthesia was given each dog. It was ascertained in some of the animals that the plasma prothrombin concentration fell mainly during the first 24 hours and in most dogs continued to fall during the second 24 hours. In other animals the tubes were disconnected and the bile collected externally in rubber balloons following the anesthesia. This bile was then analyzed for its bile salt content.* The figures are calculated in terms of cholic acid. The data are given in Table 2. It will be seen that an ample amount of bile salt very likely was entering the duodenum on the day in which the plasma prothrombin concentration fell most rapidly. On the second day bile salt production was usually impaired. It, therefore, was concluded that the observed fall was not due primarily to an interference with bile salt formation. However, interruption of the flow of bile may have some influence on the degree of the fall in prothrombin concentration as indicated by the data in Table 3. These dogs were operated upon and anesthetized in pairs. Following chloroform anesthesia the tubes of one animal of each pair were left connected to permit the flow of bile to the intestinal tract, whereas the tubes in the other animal were disconnected. In the case of Dogs 39–296 and 39–297 the experiment was repeated reversing the animals. In four out of five such

* We are indebted to Mr. Mitchell Prushankin for the bile salt determinations.

experiments the animal which received its bile showed less prolongation of the prothrombin time than did its mate which did not receive its bile.

TABLE 2.—BILE SALT CONTENT OF HEPATIC BILE COLLECTED FROM DOGS FOLLOWING CHLOROFORM ANESTHESIA.

Dog No.	Bile salt concentration first 24 hrs.* (mg. per 100 cc.).	Prothrombin time (sec.). Before anesthesia.	Prothrombin time (sec.). 24 hrs. after anesthesia.
296	4560	15	245
126	1240	14	24
194	1160	18	180

* Calculated as cholic acid.

Plasma prothrombin concentration was determined by the method of Quick (Am. J. Physiol., **114**, 282, 1936). Except in the case of the hepatectomized dogs the prothrombin times were not converted to concentration in per cent of normal. It should be remembered that prothrombin time increases as the concentration of prothrombin falls. As the concentration is reduced from 100% the prothrombin time at first increases very slowly and then more and more rapidly.

TABLE 3.—EFFECT OF INTERRUPTION OF THE FLOW OF BILE TO THE INTESTINE ON THE DEGREE OF HYPOPROTHROMBINEMIA INDUCED BY CHLOROFORM ANESTHESIA.

Dog No.	Duration of anesthesia (min.).	Bile flow interrupted.	Maximum prothrombin time (sec.).
271	30	No	18
252	30	Yes	36
296	50	No	17
297	50	Yes	45
296	50	Yes	245*
297	50	No	100*
527	55	No	27
528	55	Yes	360
536	50	No	92
537	50	Yes	23

* Prothrombin determination 24 hours after anesthesia.

Additional evidence that the major part of the fall in the plasma prothrombin concentration following chloroform is not due to the bile salt deprivation is to be found in the observation that dogs prepared as has been described would regenerate prothrombin after the first 2 or 3 days even though all bile was sidetracked from the intestine subsequent to the time of the chloroform anesthesia (Table 4).

TABLE 4.—CHANGES IN PROTHROMBIN TIME FOLLOWING CHLOROFORM ANESTHESIA IN BILE FISTULA DOGS.

	Prothrombin time (Quick*) (sec.). Dog No. 126.	Dog No. 194.
Before anesthesia	14.0	18.0
First day	24.0	180.0
Second day	50.0	43.0
Third day	21.0	19.0
Fourth day	0	0
Fifth day	0	0
Sixth day	0	0
Seventh day	14.0	
Eighth day	..	18.5

* Am. J. Physiol., **114**, 282, 1936.

That the effect of carbon tetrachloride administered orally is likewise not dependent upon an effect on bile salt production is shown in Table 5.

TABLE 5.—RECOVERY FROM HYPOPROTHROMBINEMIA INDUCED BY THE ORAL ADMINISTRATION OF CARBON TETRACHLORIDE IN A DOG WITH A COMPLETE EXTERNAL BILIARY FISTULA.

	Dog No. 126.
Dose of carbon tetrachloride (cc./kg.)	2.5
Prothrombin time:	
After 48 hours (sec.)	32
After 96 hours (sec.)	19

TABLE 6.—CHANGES IN PLASMA PROTHROMBIN IN HEPATECTOMIZED DOGS.

Dog No.	Method of hepatectomy.	Survival time (hrs.).	Preoperative prothrombin (% normal).	Last prothrombin determination before death (% normal).	Decline from preoperative level (%).
379	Mann	6	80	10	87
877	Firor and Stinson	2	No prothrombin decline*		
891	Markowitz *et al.*	1	100	32±	68
892	Markowitz *et al.*	6	82	35	57
904	Markowitz *et al.*	5¾	100	0	100
691	Mann	19	40	11	72
781	Mann	14	45	16	64
827	Mann	4	48	42†	12
				Average	58

* Dog had transfusion shortly before last specimen.
† Last specimen 2 hours postoperatively.

Two Additional Animals in Which Hepatectomy Was Performed in the Course of a Study of Cardiac Glucosides by Dr. Melvin Dresbach Also Showed an Early Prolongation of the Prothrombin Time.

	Type of hepatectomy.	Prothrombin concentration immediately after operation (% normal).	Interval before death.	Last prothrombin determination before death (% normal).
Dog D	1 stage	75	3 hrs. 45 mins.	20.0
Cat D	3 stage	77	3 hrs. 30 mins.	12.5

TABLE 7.—CHANGES IN PLASMA FIBRINOGEN IN HEPATECTOMIZED DOGS.

Dog No.	Method of hepatectomy.	Survival time (hrs.).	Fibrinogen level. Preoperative (gm./100 cc. of plasma).	Fibrinogen level. Before death (gm./100 cc. of plasma).	Decline (%).
892	Markowitz *et al.*	6	0.508	0.407	20.0
904	Markowitz *et al.*	5¾	1.019	0.400	61.2
691	Mann	19	0.297	0.229	22.9
781	Mann	14	0.805	0.779	3.3
827	Mann	4	0.511	0.226	55.8
				Average	32.6

Experiments on the Relation of the Liver to Prothrombin Formation and on the Significance of Hypoprothrombinemia Following Total Hepatectomy. We observed the effect of total hepatectomy on the plasma prothrombin level of 9 dogs and 1 cat. Most of these observations were reported in a preliminary form in a previous com-

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Third day	21.0	19.0
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Sixth day	0	0
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munication.[30] In all of these animals, except 1 dog which lived only a short time and received a transfusion shortly before the final specimen was taken, a fall in the prothrombin concentration was observed. In the dogs that lived over 4 hours, this decline was quite marked (Table 6). It was much more rapid than the accompanying decline in fibrinogen concentration (Table 7). The prothrombin determinations were carried out by the method described by Quick in 1936. As the results of this method may be influenced by severe reductions in the fibrinogen level, the effect of added fibrinogen solution prepared as directed by Smith, Warner and Brinkhous[255] was investigated. As Chart 1 shows, the results ob-

Dog 691
Effect of Added Fibrinogen on Prothrombin Determinations.

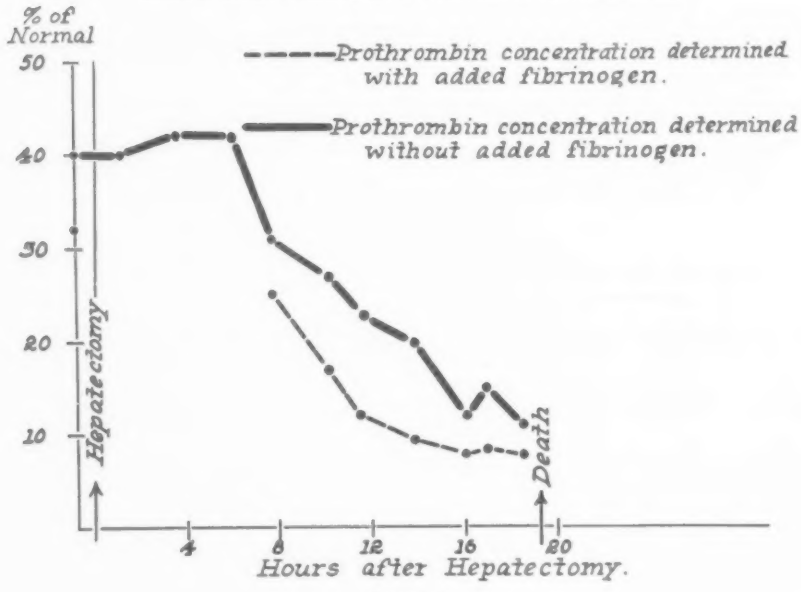

CHART 1.

tained with this modification of the Quick test for prothrombin were parallel to those obtained without the added fibrinogen. That the reduction in prothrombin concentration was not a simple dilution associated with intravenous infusions of glucose, was shown by determination of the cell volume index after centrifugation for 15 minutes at 2500 r.p.m. and by determination of the plasma protein concentration by the method of Barbour and Hamilton.[5] Slight dilution did occur but this was not of sufficient degree to account for the rapid fall in the concentration of the plasma prothrombin (Charts 2 and 3).

A number of factors, other than the removal of the liver, might

Dog 791
Changes in Prothrombin, Fibrinogen, Plasma Protein and Hematocrit After Hepatectomy.

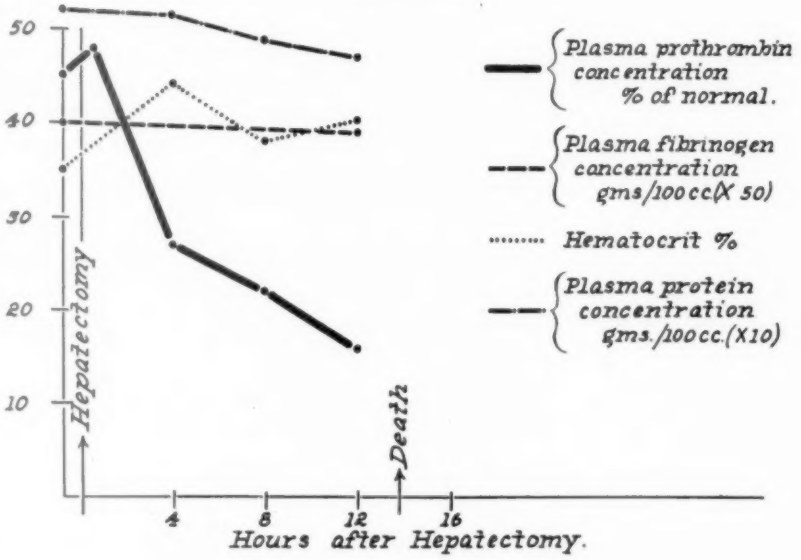

Chart 2.

Dog 691
Changes in Prothrombin, Plasma Protein, Hematocrit and Fibrinogen After Hepatectomy.

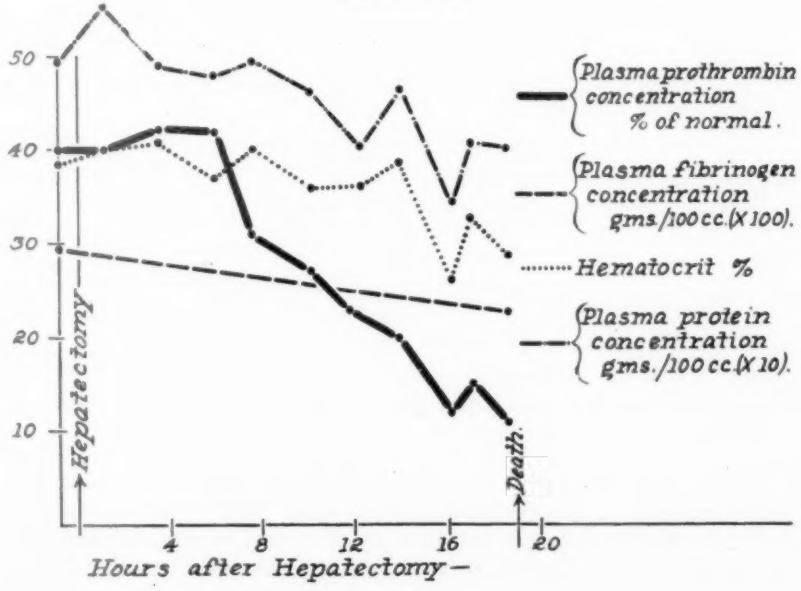

Chart 3.

have influenced the prothrombin concentration. Ether anesthesia, hemorrhage, and laparotomy were possible factors, the effect of which it seemed important to investigate. Table 8 shows the prothrombin times of dogs before and after ether anesthesia. Even when the ether was volatilized with but 15% of oxygen no striking reduction was obtained. In 1 animal in which a poor anesthetization was purposely carried out a slight fall in the plasma prothrombin concentration occurred.

TABLE 8.—THE EFFECT OF ETHER ANESTHESIA ON THE PLASMA PROTHROMBIN LEVEL.

Dog No.	Duration (hrs.).	Technique.	Remarks.	Prothrombin time. Before ether (sec.).	Prothrombin time. Maximum following ether (sec.).
237	2	Gwathmey	Atmospheric air but cyanosis maintained	13	17.5*
237	2.75	Gwathmey	15% O_2 and 85% N_2	10	10
332	3	Gwathmey	15% O_2 and 85% N_2	13	12†

* 4 days later.

† Died of pneumonia on second day.

The effects of laparotomy and operation on abdominal organs other than the biliary tract are shown in Table 9 and the effect of hemorrhage on the prothrombin level is shown in Table 10.

TABLE 9.—THE EFFECT OF LAPAROTOMY FOR OPERATIONS ON ORGANS OTHER THAN THE BILIARY TRACT ON THE PROTHROMBIN TIME IN DOGS.

Dog No.	Prothrombin time. Preoperative (sec.).	Immediately postoperative (sec.).	18 hrs. postoperative (sec.).	43 hrs. postoperative (sec.).
1	10	8	9	9.5
22	8	8	8	8
3	8	9	9	9
4	11	10.5	9	9

TABLE 10.—THE EFFECT OF HEMORRHAGE ON THE PLASMA PROTHROMBIN TIME OF DOGS.

	Prothrombin time (sec.). Dog No. 700.	Dog No. 766.
Before hemorrhage	9½	14
After 12 hours	9½	13
After 24 hours	9½	13
After 48 hours	9½	12½

In each case the dog was bled until air hunger appeared. Approximately 350 cc. of blood were removed in each case.

These three factors do not explain the fall in plasma prothrombin concentration observed following extirpation of the liver.

Interference with the absorption of vitamin K would be expected to occur following hepatectomy. The cessation of bile salt forma-

tion would certainly interfere with its absorption and the necessary alteration in the venous drainage of the intestine might likewise interfere even if bile salts from other sources were supplied. The fact that a fall in plasma prothrombin occurred after one stage hepatectomy in healthy stock dogs in whom bile salt diversion does not produce a marked drop in prothrombin for many weeks indicated that this factor was not important.

In the experiments of Andrus, Lord and Moore[3] bile salts and vitamin K were administered by enterostomy tube following hepatectomy without preventing the decline in prothrombin concentration. Despite the fact that Dam, Schoenheyder and Tage-Hansen[10] failed to decrease blood coagulability in geese by exclusion of the liver from the circulation, it seems fair to conclude that in the dog, and probably also the cat, the synthesis of prothrombin in adequate quantities requires the presence of the liver. Whether the liver completes this synthesis or whether it is responsible for only one part of it cannot be stated with the data now available.

Considerable significance may be attached to the rate with which prothrombin disappears after removal of the liver because it implies constant and rapid destruction of this factor elsewhere in the body. It makes it unnecessary to postulate that substances, such as chloroform which cause rapid drops in the prothrombin level, do so by any direct destructive action on preformed prothrombin. It also points to a discrepancy between the cessation of prothrombin synthesis caused by absence of the liver and that due to absence of vitamin K. If vitamin K were chemically combined to form prothrombin one might expect a rapid decline in prothrombin when the supply of available vitamin K was used up instead of the relatively gradual decline usually observed. So many possibilities might explain this discrepancy that further speculation at this time seems useless. However, it is well to keep in mind that prothrombin need not necessarily be a single substance but might be a mixture of two or more substances having different origins, any of which might reach critically low levels independently of the others with a resultant decline in measurable prothrombin activity.

The site of prothrombin destruction has been investigated by Andrus, Lord and Kauer.[2] After comparing the plasma prothrombin concentration of arterial blood going to various organs with that of the venous blood coming from these organs, they concluded that prothrombin was destroyed in the lungs. In 85% of the dogs they found a mean decline in the pulmonary vein blood of 10.6% when compared to pulmonary artery blood. The determinations were done by the method of Warner, Brinkhous and Smith[29] and an accuracy of 3% was claimed for the method in duplicate specimens. As the blood probably passes through the lungs at an average rate of at least 30 times an hour the observations of Andrus, Lord and Kauer[2] would indicate a more rapid destruction than we have

observed after hepatectomy. This may indicate that there are other sources of prothrombin formation.

Without finding a particular locus for the destruction of prothrombin it is possible to explain its disappearance, at least partially, on the basis of observations of the plasma prothrombin in stored blood. Although this decline is slow in blood stored at 4° C. it occurs much more rapidly in blood allowed to stand at room temperature. Blood stored in an incubator at 37° shows even more rapid changes. Chart 4 summarized certain observations on the prothrombin concentration of oxalated human plasma stored in this way. The

The Influence of Temperature on the Disappearance of Prothrombin from Stored Human Plasma.

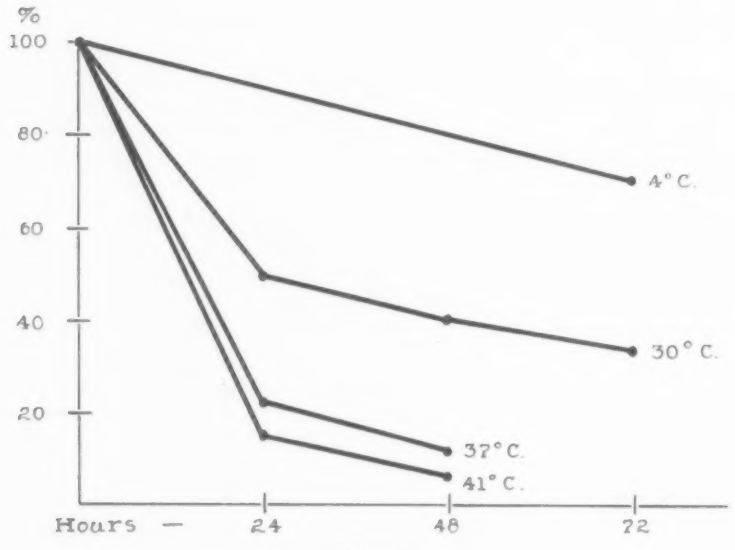

CHART 4.

decline is not as rapid as that observed in most hepatectomized dogs, but would correspond with the declines in some of the dogs observed in this laboratory.

The Effect of Vitamin K Therapy on Experimental Hypoprothrombinemia. Just as the time factor seems to vary with the mechanism by which hypoprothrombinemia is produced rather than with the particular means employed, so the response to therapy varies with the mechanism. To some extent at least the response of a hypoprothrombinemia of unknown etiology is indicative of the mechanism by which the condition has been produced.

Simple K avitaminosis in chicks responds within a few hours to the oral administration of 2-methyl-1,4-naphthoquinone (Ansbacher[4]). Vitamin K in any form will effect a prompt response.

Bile fistula animals will respond well to oral vitamin K with bile salts. The response to bile salts alone is slower but does occur.

If the absorbing membranes were at fault one would expect a poor response to vitamin K and bile salts by mouth but a good response following the parenteral administration of vitamin K. In hypoprothrombinemia produced in the dog following chloroform anesthesia, 2-methyl-1,4-naphthoquinone intravenously did not produce an appreciable effect. Andrus, Lord and Moore[3] have found that doses as large as 10,000 units of vitamin K have been without effect in influencing the prothrombin level of the hepatectomized dog. Bay[6] reports decreasing the extent of the decline in the plasma prothrombin level in the dog following chloroform by giving large doses of 2-methyl-1,4-naphthoquinone but a considerable decline occurred nevertheless.

There is, therefore, presumptive evidence that in the type of hypoprothrombinemia in patients that is due to severe liver damage, a poor response will be obtained. Histologic evidence that liver damage was present in 3 patients with hypoprothrombinemia who failed to respond to vitamin K therapy has been presented.[23a,b] Some authors have, however, expressed the opinion that by increasing the dosage of vitamin K in patients that did not respond to the usual dosage they were able to obtain a satisfactory response.[27] Investigation of this finding is difficult because the functional status of the liver may change rapidly and a sufficiently large number of clinical observations has not accumulated for statistical analysis.

The relationship between vitamin K and prothrombin is entirely obscure. They are different substances physiologically as well as chemically. Vitamin K has no activity when substituted for prothrombin in a clotting system and prothrombin preparations are only slightly beneficial when fed to K avitaminotic chicks (Dam, Schoenheyder and Tage-Hansen[10]).

If vitamin K has a mass action effect in the production of prothrombin, one would expect that quantities of it added to a normal diet might elevate the prothrombin level above normal. This hypothesis was tested in a group of miscellaneous surgical patients (Norris and Rhoads[21]) who received 6 mg. of 2-methyl-1,4-naphthoquinone daily with 15 grains of iron bile salts for varying periods of time without a significant increase in prothrombin concentration above the normal as determined by the Quick method and by certain modifications thereof. One cannot conclude from this study in relatively normal individuals that increasing the dose of vitamin K above the amount usually found adequate would not be of value in patients with liver damage but at least the failure to obtain this

response in normal persons makes one more critical of data obtained in K resistant patients with hypoprothrombinemia.

Summary. Ten means of producing hypoprothrombinemia in animals are briefly reviewed.

A comparison of experimental and clinical forms of hypoprothrombinemia point to three mechanisms for the development of hypoprothrombinemia which are common to both the experimental and clinical forms. They are a dietary K avitaminosis, a conditioned K avitaminosis due to absence of bile salts from the intestinal tract, and liver injury of sufficient degree to prevent synthesis of prothrombin at an adequate rate.

The mechanism by which the hypoprothrombinemia develops is characterized to some extent by the period required for its development. Exclusion of bile from the intestinal tract usually requires several weeks to a few months for the development of any marked degree of hypoprothrombinemia. Liver injury if it is produced rapidly is followed by a drop in the prothrombin level in a few hours. The dietary method has only been successful with regularity in poultry. An intermediate interval of 4 days to 3 weeks is required in chicks.

The three mechanisms are also characterized by the response of the hypoprothrombinemia which they produced to vitamin K. Hypoprothrombinemias due to dietary K avitaminosis are relieved by very small doses of the vitamin. Conditioned K avitaminosis due to a lack of bile salts from the intestinal tract does not respond satisfactorily to naturally occurring vitamin K in ordinary doses but will do so if bile salts are added. Hypoprothrombinemias due to hepatic damage of a degree sufficient seriously to retard prothrombin formation respond poorly or not at all to vitamin K and bile salts.

The last two mechanisms frequently operate together. Experimental evidence is presented indicating that the hypoprothrombinemia due to chloroform and carbon tetrachloride poisoning in dogs is primarily due to liver damage, although the presence of bile salts in the intestine appears to affect to some degree the extent of the fall in the prothrombin level.

Experimental data are also presented in support of the following points:

1. Chloroform anesthesia and carbon tetrachloride poisoning in dogs lead to a rapid drop in the plasma prothrombin level from which recovery occurs regardless of whether or not the bile reaches the duodenum.

2. Total hepatectomy in the dog and cat lead to a prompt decline in the plasma prothrombin level which cannot be attributed to: (*a*) hemorrhage, (*b*) ether anesthesia, (*c*) laparotomy *per se*.

3. A rapid decline in prothrombin may occur in human plasma *in vitro* at 37° C.

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THE RELATIONSHIP OF HYPOALBUMINEMIA TO THE EDEMA OF MALARIA.

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Edema occurs quite often in patients undergoing therapeutic malaria. The edema is usually regarded as of cardiac origin, provided renal damage has not developed. However, there are a series of facts which makes this hypothesis unlikely, namely: the absence of other symptoms and signs of cardiac involvement, such as increased basal pulse rate, pulmonary râles, dyspnea, cyanosis and increased venous pressure; and the rapidity with which the edema disappears with or without rest in bed, restriction of salt and fluid intake or digitalis therapy.

Other factors which must be considered as possible causes of the

dependent edema in malaria are the marked anemia, increased capillary permeability or a disturbance in the plasma proteins of the blood. The anemia of therapeutic malaria may be quite marked, as low as 2.5 million red cells, but it is unlikely that this is wholly responsible for the edema, since the latter may disappear fairly rapidly while the anemia persists. Increased capillary permeability with an increased hydrostatic pressure when the erect posture is maintained may play some part in the production of the dependent edema. It does not explain, however, the mechanism of the generalized edema. Disturbance of the colloids, especially the plasma proteins, may be an important factor since food intake is often diminished and weight losses of 10 pounds or more may occur during therapeutic malaria. A reduction in the plasma proteins, especially albumin, would change the osmotic pressure, affect the interchange of fluid between blood and tissues and thus produce edema. In order to test this hypothesis the studies described in this paper were undertaken.

Material. Seven male patients, ranging from 40 to 51 years, were hospitalized for the malarial therapy of general paresis. Physical examination and laboratory procedures revealed no other complicating diseases.

Methods. The patients had 10 to 13 paroxysms of malaria and were ambulatory between paroxysms. The malaria was terminated by the administration of 10 gr. of quinine sulphate 3 times a day for 1 week.

The daily hospital diet consisted roughly of the following: protein 80 gm., carbohydrate 315 gm., and fats 85 gm. When fever was present and food intake diminished, the daily diet was reinforced by the addition of at least 1 quart of milk and 2 eggs (protein 42 gm., carbohydrate 50 gm., and fat 52 gm.). After termination of the malaria, each patient was given a high vitamin, high caloric diet, and 20 gr. of ferrous sulphate daily. Sodium chloride was administered to 6 patients during malarial therapy as a result of the observations of Judd[7] who felt that patients were better able to tolerate the fever when 10 gr. of the salt were given on those days when body temperature rose above 103° F. Larger doses than suggested by Judd were given: 5 patients received 4 gm., and 1 patient 6 gm. daily. The seventh patient (T. G.) received no sodium chloride during his malaria other than that present in the diet. The sodium chloride was omitted when malarial fever was terminated, with the exception of 1 patient (F. F.) who continued to receive 6 gm. daily for 6 days thereafter.

Studies were made of the hematocrit, red cell count and hemoglobin (Sahli) levels on venous blood samples in the resting state. In most instances the patient was afebrile at this time.

The total plasma protein was obtained by the determination of the total nitrogen by a modified micro-Kjeldahl method;[5b] the non-protein nitrogen by the method of Folin and Wu;[3] the albumin and globulin by the sodium sulphate method of Howe;[5a] the fibrinogen by precipitation as fibrin by the method of Cullen and Van Slyke;[2] and the plasma chloride in 2 patients by a modification of the method of Van Slyke.[13] In the last series of studies (Patients F. F. and T. G.) the fibrinogen was precipitated as above, and the nitrogen content of the fibrinogen was determined directly. Potassium oxalate in the concentration of 2 mg. per cc. was used as the anticoagulant.

Results. Plasma albumin fell progressively as the malarial fever continued and even for a few days thereafter. The pre-febrile

levels of 3.7 to 5.1 gm. fell to 2.23 to 3.4 gm., and in 6 of the 7 patients were reduced to less than the critical level of 3.1 gm. at which edema is said to occur in infections.[12] The reductions represented 30% to 40% of the original levels.

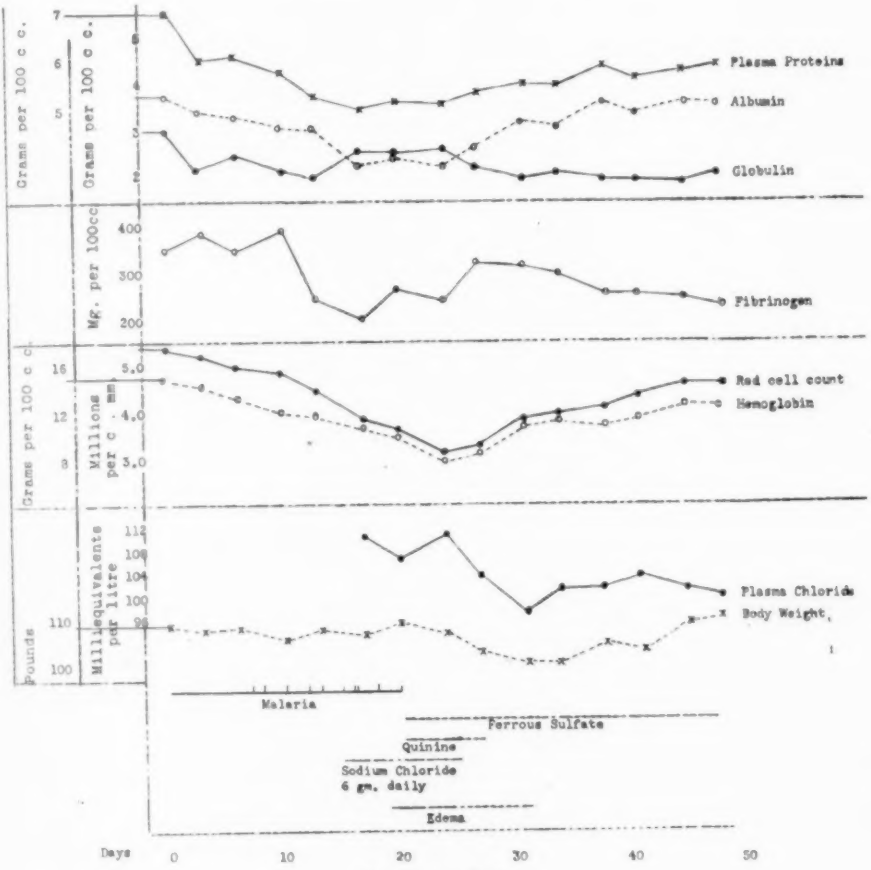

CHART 1.—F. F.

The globulin fraction, on the other hand, rose after a few paroxysms of fever, reaching its highest level (10% to 65% increase) at about the period that the albumin fraction showed its greatest decrease. The globulin changes of 2 patients differed, 1 (F. F.) showing a reduction of globulin, as great as 32%, throughout the malaria (Chart 1); and the second (T. G.) showing a progressive but less marked fall until the eleventh paroxysm (Chart 2).

Fibrinogen values did not show a consistent trend as did the albumin and globulin fractions. Considerable fluctuation occurred:

in 4 patients significant increases, in 2 patients a rise and then a fall, and in 1 patient a fall throughout.

The net result of the changes described above was a reduction of the total plasma proteins, since the increases in globulin and fibrinogen when present were insufficient to balance the reduction of albumin. Pre-febrile plasma protein values ranged from 6.88 to 8.2 gm. and fell to 5.03 to 7.3 gm., the maximum reduction being 28.5% (Patient F. F.).

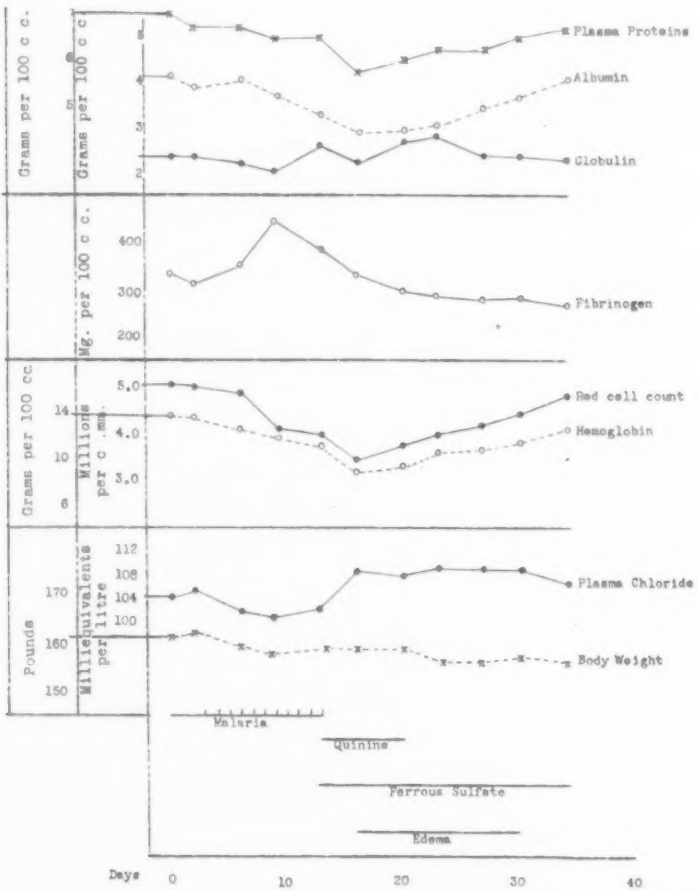

CHART 2.—T. G.

The fall in albumin ceased with the termination of the malarial fever by quinine or within a few days thereafter. The albumin rose to pre-febrile levels in 6 patients within 14 to 24 days. The return to normal in the seventh patient was quite slow, requiring

about 10 weeks. The increased globulin values, when present, persisted from 10 to 18 days. The fibrinogen returned to normal in about 1 week's time.

The albumin-globulin quotient of each patient fell during malaria: in 4 patients close to 1 and in the remaining 3 patients below 1. The quotient rose fairly rapidly toward pre-febrile levels after the termination of the malaria and as albumin increased.

The osmotic pressure of the plasma proteins (1% albumin equals 5.5 mm. of mercury: 1% globulin equals 1.4 mm. of mercury) fell progressively in each patient as the malaria fever recurred, the amount of fall depending on the reduced albumin values. Pre-febrile osmotic pressures ranged from 24.43 to 31.78 mm. of mercury and fell to levels of 15.91 to 23.04 mm. of mercury. In 6 patients the osmotic pressure fell below 21 mm. of mercury, at which level edema frequently occurs.[12] The osmotic pressure rose rapidly after the malarial fever was terminated, paralleling the increase in albumin.

Clinical edema developed in 3 of the 7 patients during malarial therapy. One patient developed dependent edema when albumin had fallen from a pre-febrile level of 5.1 to 3 gm., and while receiving 4 gm. sodium chloride daily. The edema disappeared after 10 days during which time the patient was ambulatory and the sodium chloride was omitted. The disappearance of the edema was accompanied by a rise of albumin to 4.3 gm. and a loss of 9 pounds in weight. A second patient (F. F.) developed dependent edema and edema of the face and hands when albumin had fallen from 3.67 to 2.23 gm. (Chart 1). The patient had received 6 gm. of sodium chloride daily for 4 days before the edema was noted and for 6 days thereafter. A rise in albumin to 3.2 gm. in 12 days was accompanied by disappearance of the edema, a loss of 7.5 pounds in body weight, and a fall in the plasma chlorides from 110.9 to 98.1 milliequivalents per liter (normal 99 to 106 milliequivalents). A third patient (T. G.) developed edema of the ankles and puffiness of the hands and face when albumin had fallen from 4.13 to 2.89 gm. (Chart 2). He had received no additional sodium chloride during therapy. The plasma chlorides, however, increased from 104.1 to 108.6 milliequivalents per liter when edema was first noted. The edema persisted for 14 days during which time albumin rose to 3.7 gm. but plasma chlorides remained unchanged. A fourth patient gained 2.5 pounds in body weight, but with no clinical evidence of edema when albumin had fallen from 4.37 to 2.73 gm. Albumin rose to 3.6 gm. within 3 days and body weight fell 2.5 pounds. The laboratory findings on each of the above patients revealed no evidence of nephritis as a result of the malaria.

Discussion. The important physical factors which determine the flow of fluid from the tissues into the blood stream and in the reverse direction are the osmotic and hydrostatic pressure of the fluids in

these regions.[1a] Capillary permeability and electrolytic balance, especially the sodium ion, also play important parts. The fundamental cause of edema, however, is hypoproteinemia resulting in a reduction of the osmotic pressure of the serum proteins.[11] The appearance and the intensity of the edema parallel the reduction of the albumin fraction, rather than the total plasma protein values. Moore and Van Slyke[10] found that edema was always present when albumin fell to 2.5 ± 0.2 gm. per 100 cc. of blood. Peters and Eisenman[12] found edema in more than one-half of 50 non-nephritic patients with infections when serum albumin was below 3.1%, and invariably when colloid osmotic pressure was less than 20 mm. mercury. Dehydration might prevent the occurrence of edema at these levels, whereas circulatory failure or stasis precipitated it.

Edema has been shown by some[6] to occur when colloid osmotic pressure of the plasma was reduced to levels of 17 to 21 mm. of mercury, provided hydrostatic pressure was unchanged, and by others[8] at osmotic pressures below 15 to 17 mm. of mercury. Melnick and coworkers,[9] however, point out the inadequacy of colloid pressure measurements *in vitro* and of the determination of albumin and globulin for predicting the true osmotic pressure in man. In their opinion recent physico-chemical studies have disproven the major importance of the albumin fraction in preventing the formation of edema.

The results obtained in our patients during malaria bear out the importance of plasma albumin and osmotic pressure values in the production of edema. Albumin fell to the edema level of infections, 3.1 gm. per 100 cc. of blood, in 6 of the 7 patients. The osmotic pressure of the blood of these patients fell to 21 mm. or less. Dependent edema developed in 3 patients at albumin levels of 3 gm., 2.89 gm. and 2.23 gm., and at osmotic pressures of 20.4 mm., 19.2 mm., and 15.4 mm. mercury, respectively. An increase of 2.5 pounds in body weight occurred in a fourth patient at an albumin level of 2.73 gm. and an osmotic pressure of 19.5 mm. mercury. Disappearance of the edema accompanied by loss of body weight occurred in the first 3 patients concomitant with an increase in albumin to 4.3 gm., 3.7 gm., and 3.2 gm., and a rise in the osmotic pressure to 27.9 mm., 23.8 mm., and 20.5 mm. of mercury, respectively. The absence of clinical edema in the remaining 2 patients with low albumin levels does not necessarily imply that fluid retention, subclinical in type, had not occurred and was masked by the weight changes present during malaria. Thus, though marked generalized edema occurred in 1 patient (Chart 1), the maximum body weight was only $\frac{1}{2}$ pound greater than before malaria, the accumulated fluid balancing the tissue weight loss.

It is difficult to evaluate the importance of the malarial anemia as a contributing factor in the production of the edema. Edema occurred when anemia was most marked, usually at levels of 9.7 gm.

of hemoglobin per 100 cc. of blood and 3,500,000 red cells per c.mm. or less. Not all patients, however, develop edema at these levels. Peters and Eisenman[12] observed that edema occurred at a higher level of serum albumin (4%), and at a higher osmotic pressure (26 mm. of mercury) in patients with anemia (10.9 gm. hemoglobin per 100 cc. or less). Alteration of capillary pressure and increased capillary permeability from less perfect oxygenation were thought to play some part. The low red cell count was probably of importance, since it has been shown that red blood cells *per se* produce an osmotic pressure effect.[14]

The daily intake of 4 to 6 gm. of sodium chloride during malaria is of importance, since the addition of large doses of sodium chloride (13.5 gm.) to hypoproteinemic animals frequently precipitates edema at albumin levels at which the animal was previously edema-free.[15] Edema may be produced even in healthy persons by the administration of large quantities of salt, followed by the drinking of copious amounts of water.[16] Moderately increased plasma chloride values were obtained on 2 of our 7 patients at the time of the onset of the edema, and at albumin levels of 2.89 and 2.23 gm. One patient (Chart 1) had received 6 gm. of sodium chloride daily for 4 days prior to the appearance of generalized edema and its continued daily intake thereafter for 6 days was accompanied by an intensification of the edema and persistently increased plasma chloride values. Omission of the sodium chloride was followed by a fall in plasma chloride values to normal, accompanied by a rise in serum albumin to 3.2 gm. and a rapid disappearance of the edema over a 6-day period. The second patient (Chart 2) developed an increase in plasma chlorides from 104.1 to 108.6 milliequivalents per liter, despite the fact that no additional sodium chloride was administered during malaria. This was associated with the appearance of dependent edema and puffiness of the hands and face, but to a less marked degree than in the former patient. The edema disappeared after 14 days during which time albumin rose to 3.7 gm., but plasma chloride values remained approximately the same. Though the edema of therapeutic malaria is accompanied by chloride (*i. e.*, sodium) retention, the latter is only a contributing and not the important factor in its production. Increased plasma chloride values occur in patients with suppression of urine, yet edema does not occur.[16] Our findings indicate that sodium chloride intake should be limited during therapeutic malaria, since large amounts may hasten the appearance of edema at a time when plasma albumin and colloid osmotic pressure are reduced. Nevertheless even though sodium chloride intake is limited, edema may still occur.

An additional important factor to be considered is the possible production of an hydremia due to increased sodium chloride intake. Were this so, serum protein values would diminish as a result of the increased blood plasma volume and hydrostatic pressure within

the capillaries would be increased. Veil[16] found that when large amounts of sodium chloride were taken, a rise in blood sodium chloride and a great increase in blood water first occur, followed by a pouring out of salt and water into the tissues and a return of the blood chloride value to normal. Blood plasma volume may also be increased because of the secondary anemia present in malaria.[4] The blood plasma volume of patients with Epstein's nephrosis, however, is diminished.[4] In this condition plasma proteins, especially albumin, are considerably reduced, serum chlorides are above normal if altered at all and edema may be generalized and marked. The low plasma albumin and osmotic pressure values of patients during malaria are factors against the presence of a significantly increased blood plasma volume. Increased hydrostatic pressure does not occur, since signs of increased venous pressure are not seen.

Conclusions. 1. Marked disturbances with a reduction of the plasma proteins occur in malarial fever. Albumin values fall progressively to critical levels at which edema may occur. Globulin, as a rule, shows a progressive increase after the first few paroxysms, reaching its highest level at a time when albumin values are about lowest. Fibrinogen values fluctuate considerably and in 3 of 7 patients were reduced below pre-febrile levels.

2. The termination of malarial fever is followed by an immediate and continued rise of albumin and a delayed but progressive drop of globulin from its highest level so that normal values are obtained in from 10 to 24 days. Fibrinogen values return to normal within 1 week's time.

3. The albumin-globulin ratio falls rapidly during malaria reaching levels of 1 or below after 10 to 12 paroxysms have occurred.

4. The dependent or generalized edema occurring during the course of therapeutic malaria in patients free from renal damage or cardiac failure, is the result of a reduced osmotic pressure caused by a marked fall of the albumin fraction to levels of 3 gm. per 100 cc. or below.

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THE SIGNIFICANCE OF TARGET CELLS IN ANEMIA.

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In 1937 Haden and Evans[4] described what they termed "dimpled" cells in the stained blood smear. The following year Barrett[1] described these cells more fully and showed that when in the circulating blood the cells were bowl-shaped; that in the process of drying they formed a hump in the center, giving rise to what he appropriately named "target" cells. He also discovered that the target cells are very resistant to hypotonic salt solutions *in vitro* and noted their occurrence in anemias associated with obstructive jaundice and in severe hypochromic anemia. Haden and Evans had observed them in small numbers in various anemias but in large numbers in sickle-cell anemia.

Their increased resistance (decreased fragility) in hypotonic solutions has been confirmed by Dameshek,[3] who found target cells in Cooley's anemia, and by me. In my case[2a] increased resistance was exhibited also to acetic acid, so that a leukocyte count could not be made until the acetic acid of the diluting fluid was increased to 3%.

The nature of the target cell has not yet been elucidated and in the light of Dameshek's paper is likely to be disputed. Dameshek reported an anemia resembling Cooley's anemia with as many as 32% of the erythrocytes target cells, which he designated as a "target cell" anemia, though he also found them in other anemias. He regards the target cell as a congenitally defective cell, possibly the fundamental defect in Cooley's anemia, and points out that a cell which is hyperresistant *in vitro* may be hyporesistant *in vivo*. Certain observations of my own, however, incline me to the view that the target cell is a newly formed cell, produced by the bone marrow in response to blood loss regardless of the cause.

In the first place, the appearance of target cells in the stained blood film, far from being an unusual event, *is very common*. Such cells can be found in small numbers, up to 1% or 2%, *early* in the majority of cases of anemia. Rare ones may even sometimes be seen in apparently "normal" smears. In more chronic anemias the number is variable but they may be found in from 1% to 10% or more in many cases.

The presence of the target cells in stained smears is transitory in acute anemia. Often they disappear before there is a significant rise of the erythrocyte count, in this way resembling the reticulocyte. On several occasions they have been present for only 1 or 2 days. Rarely are they present for as much as a week, while in the majority of cases they are seen for 4 or 5 days. They usually first appear on the third or fourth day following a hemorrhage,

although in 1 case they were first seen on the day after a hemorrhage from peptic ulcer. Coincident with their appearance there is a transitory decreased fragility of the erythrocytes; the latter phenomenon is well known. The following case illustrates the course of events:

Case Abstract. CASE 1.—A woman, aged 23, lost considerable blood during and following the delivery of her second child. On the second postpartum day her erythrocyte count was 3.4 million, her hemoglobin 67% (Sahli). Color index 0.98. There were no target cells, the stained smear appeared normal. The next day a few polychromatophilic erythrocytes were present and some of these were target cells. On the fourth and fifth postpartum days polychromatophilic cells were more numerous (between 1% and 2%) and nearly every one was a target cell. None of the normally stained cells were target forms. In the next few days the number of polychromatophilic cells diminished, as did the target cells and a few target forms were seen in acidophilic cells. The important point is that *at one stage only those cells showed the target form which by their polychromatophilia indicated their recent formation in response to the blood loss.* By the time of the patient's discharge from the hospital on the twelfth postpartum day the erythrocyte count was 4.1 million, hemoglobin 76%, color index 0.92. The stained smear appeared normal; there were no polychromatophilic cells and no target cells.

In most cases, target cells are seen only after a moderately severe hemorrhage. Occasionally the loss of blood is slight, as the following case illustrates.

CASE 2.—A woman received a laceration of the scalp in an automobile accident. Bleeding was, according to her, profuse, but it was stopped in about 45 minutes. No blood count was made until the fourth day later, when it was found to be 4.5 million; 2% of the erythrocytes were target cells. Three days later target cells had disappeared although the erythrocyte count had not changed.

Target cells are usually of normal diameter. In the stained smear they are made up of a rim of hemoglobin-containing material and a central hemoglobin-containing area, the two areas being separated by a zone which is clear, unstained. Barrett conjectured that this appearance was due to a central knob-like projection in a cup or bowl-shaped cell, and showed that a body of such shape would look like a target when viewed on the broad surface. That this is actually the shape of the cell can be seen by oblique illumination of dry smears[2b] or in bas-relief photographs (Fig. 1). Variations from the typical target form are seen in every smear which exhibits the characteristic appearance. The central stained mass may be joined to the outer rim by a bar of varying thickness; or a ridge may cross the center of the cell, with a thickened central dot. These variations are probably the result both of viewing the dimpled, cup-shaped cell from varying angles and of differences in the distortion of the individual cell.

No target cells are seen in wet preparations. Instead, a number of cells, equivalent to the proportion which appear as target cells

in the stained smear, appear as shallow or, more uncommonly, deep bowls or cups. Rarely, the bowl shape is preserved in the dry smear (upper part of Fig. 1). When blood is diluted with hypotonic salt solutions of varying concentrations the proportion of bowl-shaped cells (or of target cells in dry smears) increases as the salt concentration decreases. This is due to the hemolysis of the normal appearing cells and the preservation of the target cells. Even in distilled water a few target cells may resist hemolysis, and most of them resist hemolysis in 0.3% NaCl. The target cells are resistant to acetic acid in concentrations which destroy normal cells.[2a]

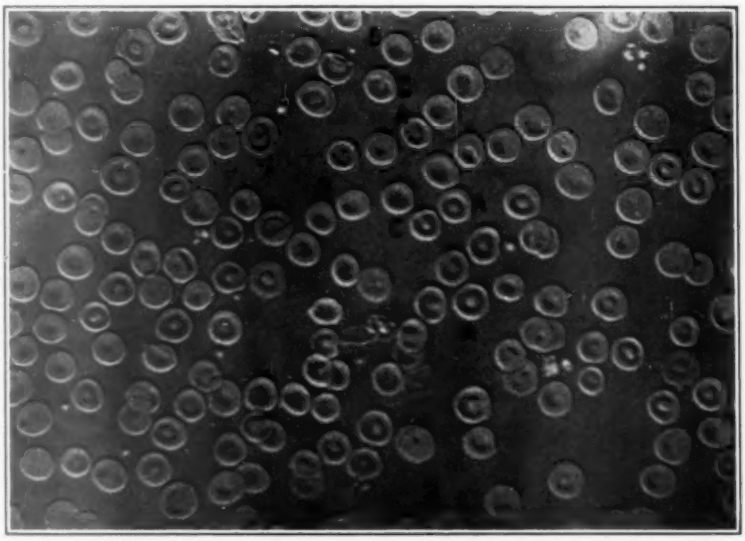

FIG. 1.—Bas-relief photomicrograph of target cells, made by printing through superimposed positive and negative films. Cover-slip dry smear, Wright stain. Some target cells, marked *A;* a bowl-shaped cell, marked *B;* an intermediate stage, *C*. Cells which maintain their bowl shape in dry smears are of rare occurrence.

There seems to be little reason for assuming that the target cell is a fundamentally deficient cell in any type of anemia. Certainly neither the frequency of occurrence nor the number of such cells in Cooley's anemia is any greater than they are in sickle-cell anemia or in cases of acute blood loss. The common occurrence of target cells, if watched for, in the early phases of regeneration of blood does not indicate that this cell is congenitally malformed in the same sense as the sickle cell, the elliptical cell, or the spherocyte. The target cell is merely a hyperresistant young cell which appears in the blood following loss of blood. If the loss is chronic the cells may be present constantly and in large numbers. Its occurrence is analogous to that of the reticulocyte, which also appears in the

blood stream early in the regeneration of erythrocytes and has practically disappeared by the time the red blood count is well on its way to normal. In chronic recurrent blood destruction, as in congenital hemolytic anemia, the reticulocyte counts may be persistently elevated. It is perhaps significant that in Dameshek's "target-cell anemia" there was an increased number of reticulocytes, and that the only one of the patient's relations who showed target cells also had a reticulocytosis.

The one stumbling block to the ready acceptance of this simple concept of the target cell is the oddity of the shape of the cell. Why should the regeneration of blood be associated with a change of shape of the erythrocyte? With one assumption, namely, that the cell does *not* change its shape *in vivo*, it is possible to explain the appearance of the target cell. This assumption is not as startling as may at first sight appear.

The shape of the *circulating* erythrocyte is unknown. While the majority of authors accept the biconcave disc seen on slides as the normal shape of the cell, there have long been dissenters to this view. Weidenreich[7] was perhaps the first to state that the normal form of the erythrocyte was a "bell-form," basing his claim upon the observation of cells in the mesentery. Radasch[6] examined sections of placenta and also noted a bowl or bell form, and he supposed that the biconcave form was due to contact of the cells with the air. Repeatedly, bowl-shaped cells have been noted in wet smears of apparently normal blood and in sections of normal tissues. Maximow and Bloom[5] summarize the status of this question: "Although the fresh human erythrocytes are one of the most common objects of microscopic study, the question of their natural form in living conditions in the circulating blood is still unsettled. Some investigators claim that in reality they are not biconcave discs, but thick-walled, shallow cups, that is, convex-concave discs, and that the biconcave form is merely the result of shrinkage due to an increase in the osmotic pressure of the plasma during examination. It is possible that in the normal blood both forms, as well as all transitions between them, exist at the same time."

I have repeatedly noted cup-shaped cells in histologic sections of blood clots, most of the time in the center of the clot, where presumably there was no contact with the air. It is a simple matter to collect blood with little or no contact with the air, but it is not so simple to achieve the examination of a sufficiently thin film under the microscope without allowing contact with air. Some preliminary experiments along these lines have demonstrated the presence of significant numbers of bowl-shaped cells in normal blood, and these experiments are now being continued.

If the bowl-shaped cell is the normal form of the erythrocyte, the biconcave disc is the result of the deforming influence of the atmo-

sphere.* A change in shape of the cell after it is removed from the body is not a new concept. The sickle cell is not sickle shaped within the blood stream but becomes so when exposed to increased carbon dioxide tension. There is reason to suppose[2c] that the eccentricity of the elliptical cell increases after removal from the body. In line with this assumption, the target cell is one which resists the action of the atmosphere toward biconcavity and maintains a modification of its normal intravascular shape. Its increased resistance to the atmosphere is correlated with its increased resistance to the hemolytic action of hypotonic solutions and to acetic acid.

It should be pointed out that, however valuable these increased resistances may be for the recognition or isolation of the target cells, the functional value of the hyperresistant cells must lie in some other form of increased resistance not yet recognized. The normal cell within the body is not exposed to the atmosphere, nor to solutions of markedly lowered osmotic pressure, nor to acids. Its reactions are the opposite of those of the spherocyte. The laboratory examination of this latter cell utilizes its diminished resistance to hypotonic salt solutions, but it is merely an indication and not the property of the cell which is responsible for the increased hemolysis *in vivo;* for the blood in congenital hemolytic anemia, or in any other known condition, never reaches the degree of hypotonicity which would hemolyze the abnormal cells.

No evidence has yet been presented to support the contention[3] that the target cell, hyperresistant *in vitro*, is *in vivo* more friable than the usual erythrocyte, and that the destruction of such cells leads to a hemolytic anemia, Cooley's anemia. Until such evidence is forthcoming, it is simpler to conceive of these cells as produced following the increased destruction of blood. In Cooley's anemia the cause of this destruction is as yet unknown, and no fundamental defect of the erythrocyte can yet be said to have been demonstrated.

Conclusions. 1. Target cells are commonly seen in the regeneration of blood, regardless of the cause of the blood loss.

2. In acute anemias they are present for only a short time early in the regenerative phase, and they have disappeared by the time a significant rise of erythrocyte count is evident. In chronic anemias they may be present over long periods of time.

3. The question of the normal shape of the circulating erythrocyte should be reëxamined. It is possible, but not yet proven, that the target cell preserves the bowl shape of the intravascular cell in modified form and resists deforming influences which are responsible for producing the biconcave disc seen in blood outside the body.

4. The target cell is a hyperresistant cell produced by the bone marrow in response to the blood loss. Increased resistance to the

* The word atmosphere is here used very loosely to include all those influences which may affect the blood cells once they are removed from the body.

hemolytic action of hypotonic saline and to acetic acid has been demonstrated.

5. The contention that the target cell represents the fundamental defect in Cooley's anemia seems to be unjustified. It is simpler, in the light of available evidence, to regard the target cell in this disease, as in sickle-cell anemia and many other anemias, as a response to the blood destruction rather than the cause of it.

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TWO CASES OF STAPHYLOCOCCIC MENINGITIS TREATED WITH ASPARAGIN BACTERIOPHAGE.

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Recoveries from meningitis in which the *Staph. aureus* is the offending organism are sufficiently uncommon to suggest that the therapeutic agent employed in a successful case may have been responsible for the favorable outcome. Survival of patients with this highly fatal lesion has been reported following the use of various procedures and combinations of therapeutic agents. Up to February, 1941, there had been reported in the American and British literature 15 such recoveries. The treatments used in these cases were as follows:

Case 1: Repeated transfusions of blood and surgical drainage of the cisterna magna.[8]

Case 2: Sulfanilamide by mouth.[3]

Case 3: Sulfapyridine by mouth.[7]

Case 4: Repeated lumbar punctures, cisternal tap, blood transfusions, intravenous fluids, staphylococcus antitoxin intravenously and intrathecally, and sulfanilamide intrathecally and by mouth.[14]

Case 5: Staphylococcus antitoxin intrathecally, intramuscularly, and "Prontosil" intramuscularly.[6]

Case 6: Lumbar puncture, blood transfusions, antimeningococcus serum, and sulfanilamide.[5]

Case 7: Intraventricular gentian violet.[9]

Case 8: Intravenous gentian violet.[2]

Case 9: General supportive measures. No "specific" treatment.[1]

Case 10: Antimeningococcus serum intrathecally, antistreptococcus serum plus 1:4000 dilution of acriflavine intrathecally, 5 cc. of commercial bacteriophage preparation plus 1:4000 acriflavine intrathecally, and (following isolation of the specific organism) 5 cc. of commercial bacteriophage intrathecally at 24-hour intervals for four doses.[4]

Case 11: Broth bacteriophage intrathecally.[17]

Case 12: Broth bacteriophage intrathecally.[17]

Case 13: Sulfanilamide, blood transfusions, and repeated lumbar punctures.[12]

Case 14: Broth bacteriophage intrathecally.[16]

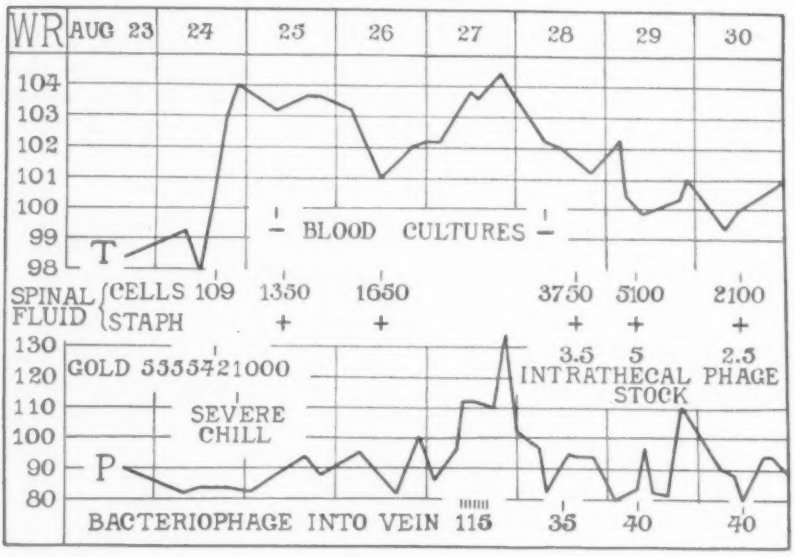
Case 15: Sulfathiazole intravenously, subcutaneously, by mouth, and intrathecally.[15]

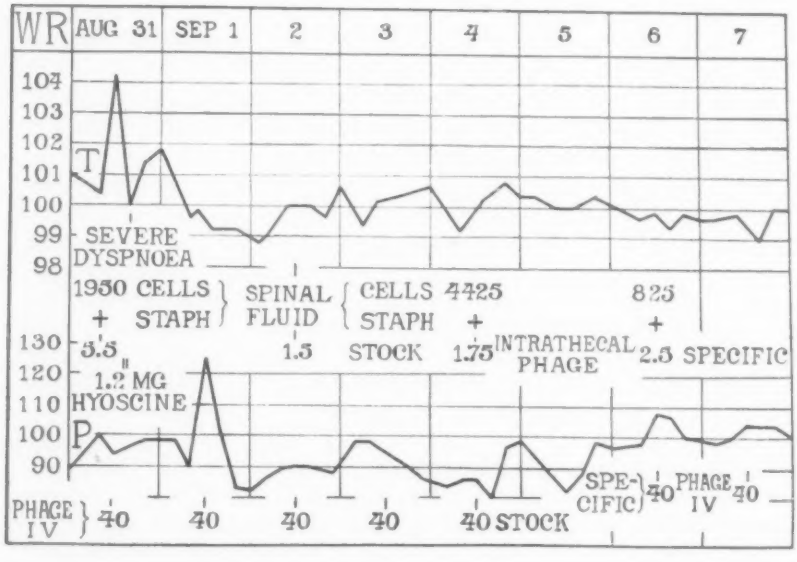
In order to gain additional insight into this problem the records of all patients with bacteriologically proven *Staph. aureus* meningitis seen at this hospital from 1925 through 1940 were reviewed. Of these, there were 17. One patient who received massive doses of staphylococcus antitoxin (320,000 units), and sulfapyridine by mouth until the blood level reached 8 mg. per 100 cc., was discharged as cured, but personal investigation by one of us (D. B. F.) discovered that this patient suffered a relapse at home and succumbed to the infection.

During this study there was discovered a large group of patients on whom the diagnosis of "purulent meningitis" had been made but bacteriologic proof of the exact etiologic factor had not been obtained. In these cases culture of the cerebrospinal fluid either was not performed or showed no growth. In all cases, however, the spinal fluid was described as "purulent" and the clinical syndrome did not suggest luetic, tuberculous or "sterile" meningitis. These cases were also studied because of the possibility that in some of them the staphylococcus might have been the infecting organism. In this group there were 81 patients of whom one survived. This was a 16-month-old infant with an infected myelomeningocele whose spinal fluid contained "excessive numbers" of leukocytes. Bacteriologic examination was not performed. Following surgical excision of the myelomeningocele recovery was prompt.

The mortality rate, therefore, in the 17 cases of bacteriologically proven staphylococcic meningitis was 100%, and in the "purulent" group was 98.7%. From the fact that only 15 recorded recoveries could be readily found in the English literature we believe that it may be safely assumed that the general mortality rate from staphylococcic meningitis does not vary to any great extent from the above figures. It is our purpose here, therefore, to describe the treatment of 2 patients suffering from this disease by means of

asparagin bacteriophage* and to add the recovery of one of them to the list of 15 already recorded.





* The bacteriophage used in the treatment of these patients was supplied by the Department of Bacteriology of the New York Post-Graduate Medical School and Hospital, Columbia University, New York City.

In the selection of a therapeutic agent to be used in the treatment of these patients we followed the advice of Josephine Neal: "In cases of meningitis due to the Staphylococcus . . . . bacteriophage should be used if available."[13] The bacteriophage used was prepared in asparagin medium suitable for intravenous injection according to techniques previously described by others.[11] It was given in full strength into the vein but for intrathecal injection was diluted 1:4 with 0.4% saline solution in order that the resulting solution might be hypotonic.

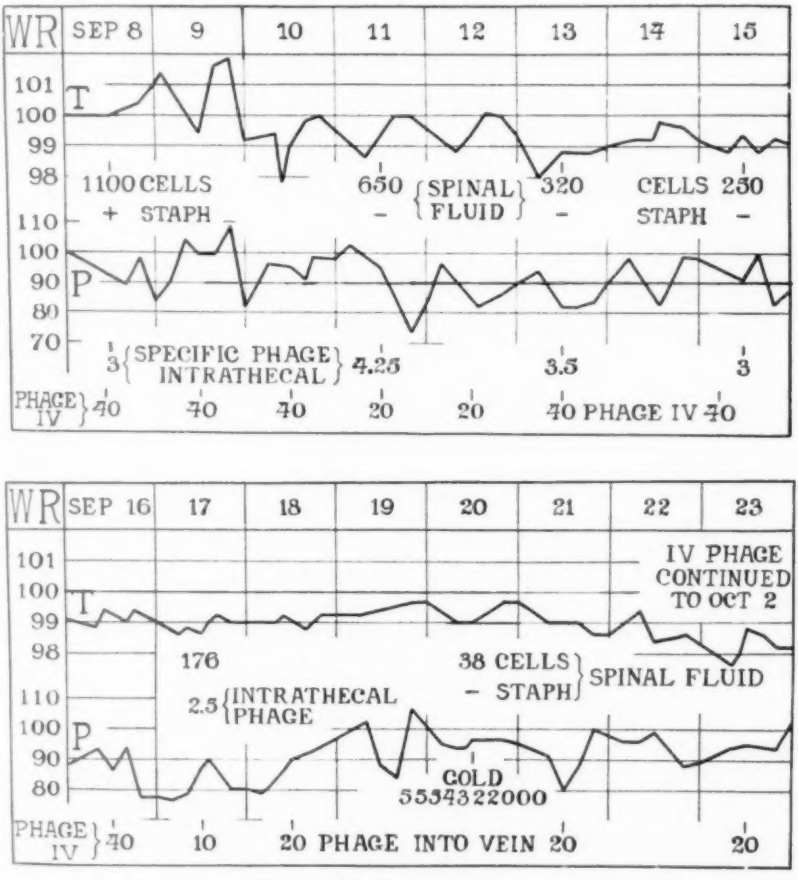


CHART 1.

Case Reports. CASE 1.—W. R. (Univ. of Michigan Hosp. No. 448868), a 41-year-old white male, was admitted to the ward with the diagnosis of syphilis of the central nervous system on Aug. 23, 1939. The following day, Aug. 24, a diagnostic lumbar puncture was performed despite the fact

that there existed a small pustule about 5 cm. from the site chosen for insertion of the needle. The fluid removed at this time showed 109 lymphocytes per c.mm. and a gold sol curve of 5555421000. Within 4 hours following this procedure the patient suffered a shaking chill with rise of temperature to 104° F. Lumbar puncture the following morning (Aug. 25) revealed 1350 cells per c.mm., nearly all of which were neutrophils. At this time it was noted that nuchal rigidity and Kernig's sign were present. Culture of the fluid taken at this time revealed, 36 hours later, a profuse growth of *Staph. aureus*. Before the bacteriologic report was available sulfanilamide was begun by mouth, 1 gm. every 4 hours. This was administered on Aug. 26 and 27. When the bacteriologic examination revealed that the noxious microbe was the staphylococcus, bacteriophage therapy was begun at once and the sulfanilamide discontinued. Because of the possibility of a concurrent septicemia, a blood culture was taken and intravenous bacteriophage begun at the same time according to a dosage schedule previously outlined.[10] Thus, on Aug. 28, 115 cc. were given intravenously in divided doses and 14 cc. of the 1:4 dilution with 0.4% saline were administered intrathecally (3.5 cc. of actual bacteriophage). On Aug. 29, and subsequent days until Sept. 4, 20 cc. were given intravenously morning and afternoon. Larger doses were not employed because the blood remained sterile. The intrathecal dose varied from day to day, depending in part on how much of the spinal fluid could be withdrawn; in general, about two-thirds of the amount of fluid removed was replaced with the hypotonic dilution of bacteriophage. Thus the patient received intrathecal doses of 1:4 dilution in the amounts of 20 cc. on Aug. 29, 10 cc. on the 30th, 22 cc. on the 31st. None was given on Sept. 1, but the patient received 6 cc. on the 2d, 7 cc. on the 4th, and 10 cc. on the 6th. On Sept. 1 rather large doses of hyoscine were given in an effort to control acute mania and this was followed by marked reduction in respiratory rate, fall in blood pressure, and deep cyanosis. However, he rallied from this "low." On Sept. 8th he received 12 cc. intrathecally; on the 11th, 17 cc.; on the 13th, 14 cc.; on the 15th, 12 cc.; and on the 17th his final intrathecal injection of 10 cc.

Laboratory findings revealed negative blood cultures on Aug. 25 and 28. Cultures of the spinal fluid revealed pure growth of *Staph. aureus* on Aug. 25, 26 and 30. No growth occurred on specimens obtained on Sept. 2, 14 and 20. Stained smears of the spinal fluid revealed Gram-positive cocci in clumps on Aug. 25, 26, 28, 29, 30, 31, and on Sept. 4, 6 and 8. No organisms could be seen on Sept. 11, 13 or 15. The patient was discharged from the hospital as cured 30 days after his original admission.

This patient had been admitted originally with the intention of administering malarial hyperpyrexia as treatment for the luetic involvement of the central nervous system. It is interesting to note that 2 months after his bout of fever due to the meningitis he returned to the hospital feeling well and 20 pounds heavier than he had been previously. Malarial therapy was subsequently administered without mishap. The clinical course of the meningitis is outlined in the accompanying chart.

Case 2.—R. T. (Univ. of Michigan Hosp. No. 270059), a 20-year-old white male, had first been seen here in July of 1931 at the age of 11, suffering from acute osteomyelitis of the femur. Over the next 8 years, despite repeated surgical intervention, the process spread to involve the left femur, right metatarsals, right ulna, left radius, right femur, both mastoid areas, left tibia, right humerus, left metatarsals, left fibula and tibia, left humerus, and the left knee. Each of these areas had been treated surgically on at least one occasion. Cultures of the pus from each site had shown pure growth of *Staph. aureus*. Throughout this period repeated blood cultures had always been negative.

On Sept. 22, 1939, the left ulna was incised and drained because of further extension of the osteomyelitic process. The evening of that day he developed left facial palsy of the peripheral type. On October 2 the left ear began to drain and paracentesis of this drum was performed in an effort to improve drainage. On October 6 definite signs of meningitis developed and on the following day a radical mastoidectomy was performed on the left side. Because all previous cultures from wounds had yielded *Staph. aureus* it was thought likely that the meningitis was due to this same organism and treatment was begun with asparagin bacteriophage in divided doses intravenously[10] on the day of the mastoidectomy (Oct. 7), the patient receiving a total of 50 cc. on this date. Lumbar puncture was postponed to the following day, due to the inability of the patient to coöperate. On Oct. 8, therefore, he received 17 cc. of the hypotonic solution intrathecally. With but slight variation this dose was repeated daily through Oct. 16, the day of the patient's death. In addition, he received 20 cc. morning and night by the intravenous route. The initial culture of the spinal fluid taken on Oct. 6 showed pure growth of *Staph. aureus*. On Oct. 10 a report was received that the fluid drawn the day before contained not only the staphylococcus but also many colonies of *Strep. viridans*. Because of this fact and the realization that bacteriophage was not effective against the streptococcus, sulfapyridine was administered by mouth in doses of 1 gm. every 4 hours from Oct. 10 to Oct. 15. Under this combined therapeutic regimen the spinal fluid became sterile on Oct. 11 and additional cultures obtained on Oct. 12, 13 and 14 revealed no growth. The patient failed to realize this, however, and his course continued downhill until he ceased to breathe on Oct. 16. Postmortem examination was not permitted.

Summary. 1. Recoveries from meningitis due to the *Staph. aureus* are uncommon—the general rate of mortality being in all likelihood between 98% and 100%.

2. Reference is made to 15 cases of this disease followed by survival of the patient, and the treatment employed in each is briefly mentioned.

3. Two cases treated with asparagin bacteriophage intravenously and intrathecally are presented. The recovery of one of these is noted and the treatment outlined in detail.

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NOTE ON LOCALIZED LIPOID ATROPHY IN DIABETES.

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Numerous cases of localized fat atrophy at the site of insulin injections in diabetic individuals have been described by various authors since the first cases were reported by Barborka[1] and Depisch[4] in 1926. The atrophy is usually first observed about 3 months after insulin administration is instituted, although it may appear later. The great majority of the cases reported in the literature have occurred in women and children, the lesion being quite rare in adult males. In a diabetes clinic with an enrollment averaging about 300 patients, we have seen only 2 cases of fat atrophy in a period of 4 years, 1 in a young girl and the other in an adult female.

The etiology of this condition remains obscure, although many theories have been proposed by the various authors who have reported cases. However, disappearance of the atrophy has been reported by several authors. Chapman[3] observed disappearance of the lesion in a patient 2½ years after omitting insulin entirely. Nichols[6] reports regeneration of fat during a period of 3 years after changing the site of injections. The general conclusion of all authors, regardless of etiologic theories, was that repeated injections in a localized area should be avoided in order to prevent the occurrence of fat atrophy following insulin administration. That recovery may take place without variation of the site of injection is demonstrated by one of the cases which came under our observation.

Case Report. L. G., female, age 10 years, was first admitted to this hospital on January 26, 1938, in acidosis. She recovered after the usual treatment and was discharged 16 days after admission on a diet of 60 gm. protein, 200 gm. carbohydrate, and 50 gm. fat. A single dose of 40 units of protamine zinc insulin in the morning sufficed to control the glycosuria satisfactorily throughout the day.

Due to poor coöperation at home and to the somewhat subnormal mentality of the patient, the prescribed diet was not followed closely and, as a result, the patient had frequent periods of marked glycosuria. The addition of a dose of regular insulin did not improve the degree of control obtained. Following her return from a private diabetic camp in August, 1938, approximately 7 months after insulin was first administered, fat atrophy over both upper arms was first observed (Fig. 1). She had had no swelling, redness or pain at the site of injections in the arms at any time. Her mother was advised to transfer the site of injections to the thigh which had not been used heretofore.

On August 24, 1938, the patient was hospitalized in mild ketosis which rapidly cleared up under treatment. At this time she was receiving 1 dose of protamine zinc insulin and 2 doses of regular insulin in her thighs. The atrophy in the arms had shown slight progress.

FIG. 1.—Marked lipoid atrophy (7 months after first administration of insulin).

FIG. 2.—Recovery from localized fat atrophy in spite of continued injection of insulin in and around the involved area.

Fat atrophy in both thighs was first observed on May 17, 1939. The condition of the upper arms had shown no improvement at this time but had continued to progress although no insulin was being given in the arms at this time. It was decided to continue injections in the thighs and arms in order to determine if recovery would occur even though injections were continued in and around the areas of atrophy. During the following year the degree of control of diabetes in this case improved considerably, although not to the extent of being entirely satisfactory for any prolonged period of time. Nevertheless, gradual and complete restoration of subcutaneous fat in both arms and thighs occurred. The appearance of the arms on June 26, 1940, is illustrated in Figure 2. This restoration of subcutaneous fat was

associated with growth of the child and with considerable normal fat deposition elsewhere, as noted in the photograph. During this period of 1 year the patient gained over 10 pounds in weight.

Certain significant problems present themselves when one considers the etiology of subcutaneous fat atrophy following the injection of insulin. If the sole cause of the atrophy is the repeated injection of insulin in one area, the rarity of the lesion is certainly remarkable.

It is quite safe to surmise that especially in the average outpatient department, where patients who disregard instructions are not uncommon, there are many diabetics who inject their insulin within the confines of a relatively small area. Yet, Rosenberg and Berliner[7] observed fat atrophy in only 32 out of 4000 diabetics receiving insulin, an incidence of less than 1%. It is highly probable that such factors as inflammation or cell injury are not the sole factors involved, since these undoubtedly occur after the injection of substances other than insulin. That the presence of diabetes is not essential to the occurrence of atrophy after insulin injection is demonstrated by a case described by Blotner[2] in which insulin injections were administered to a non-diabetic in the treatment of undernutrition. Fat atrophy identical in appearance to that occurring in diabetics after insulin administration developed in this patient.

It appears quite likely that some intrinsic factor in certain patients makes them susceptible to this condition and that when this factor is eliminated, spontaneous recovery occurs. Joslin[5] states that the condition occurs exceptionally in patients whose diabetes is controlled. It is quite probable that this plays a significant part (as in the present case), but in view of the rarity of this condition among diabetics including those poorly controlled and its occurrence in non-diabetics, another factor in fat metabolism undoubtedly plays an important rôle.

Conclusion. Although repeated injection of insulin within a restricted area is not advisable on general principles, this case appears to indicate that recovery from localized fat atrophy may occur in spite of continued injection of insulin in and around the involved area.

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BOOK REVIEWS AND NOTICES

ELIMINATION DIETS AND THE PATIENT'S ALLERGIES. A Handbook of Allergy. By ALBERT H. ROWE, M.D., Lecturer in Medicine, University of California Medical School, San Francisco, California; Consultant in Allergic Diseases, Alameda County Hospital, Oakland, California. Pp. 264. Philadelphia: Lea & Febiger, 1941. Price, $3.00.

ELIMINATION diets have proved a valuable aid in the diagnosis and treatment of food allergy. As their leading exponent, the author of this volume presents yet further improvements in their application, based on a wider experience and accumulated knowledge. Important modifications include a fruit-free diet, a cereal-free diet, diets without cottonseed oil and shortening, elimination diets for diabetics, for the young, the obese and the undernourished. Detailed instructions are given, including sample menus and recipes. In addition there are chapters on the diagnosis of allergy in general, its manifestations and its control, furnishing an adequate clinical survey of the subject. In the appendix is much practical information, including questionnaires and history forms for eliciting the history in cases of allergy; food diary forms; directions for the administration of pollen antigens; a botanical classification of edible plants; a list of the ingredients of many commercial foods; the vitamin content of foods; and many recipes. The chief characteristic of the book is its highly practical clinical approach, which should recommend it both to practitioners and allergists. R. K.

THEORY OF OCCUPATIONAL THERAPY FOR STUDENTS AND NURSES. By NORAH A. HAWORTH, M.A. (CANTAB.), M.R.C.S., L.R.C.P., D.P.M., Late Senior Assistant Medical Officer, Severalls Mental Hospital, Colchester, and Honorary Assistant Physician, Lady Chichester Hospital for Functional Nervous Diseases, Hove, and E. MARY MACDONALD, Principal, Dorset House School of Occupational Therapy, and Occupational Therapist in Charge of the Allendale Curative Workshop, Bristol, with a Foreword by SIR ROBERT STANTON WOODS, M.D., F.R.C.P., Consultant Adviser in Physical Medicine to the Ministry of Health, etc. Pp. 132; 81 illustrations. Baltimore: The Williams & Wilkins Company, 1941. Price, $2.00.

AN elementary and concise statement of the uses and methods of application of occupational therapy, this book was written for nurses and for students of the subject, but it would prove instructive and stimulating reading for many physicians. Psychiatrists and orthopedists have of course long known the therapeutic value of productive activity, but many internists, surgeons and other members of the staffs of general hospitals give too little thought to this therapeutic measure, and would do well to obtain an orderly viewpoint of the subject by browsing through this little volume. It discusses occupational therapy in the treatment of mental disorders, in mental nursing, in the treatment of tuberculosis and cardiac disease, in the treatment of surgical and orthopedic cases; and considers the problems of finance, equipment, stocking, storage and use of waste materials in occupational therapy. There are appended drawings and descriptions of suitable apparatus; lists of suppliers (British) of materials and apparatus; and a considerable bibliography of all aspects of the subject. R. K.

CARDIAC CLINICS. A Mayo Clinic Monograph. By FREDRICK A. WILLIUS, B.S., M.D., M.S., in Med., Head of Section of Cardiology, Mayo Clinic, and Professor of Medicine, Mayo Foundation for Medical Education and Research, Graduate School, University of Minnesota, Rochester, Minn. Pp. 276; 35 figures. St. Louis: The C. V. Mosby Company, 1941. Price, $4.00.

ACCORDING to the author's foreword: "These brief informal discussions appeared without special order of subject matter in the Proceedings of the Staff Meetings of the Mayo Clinic. . . . The guiding idea was a desire to present concise practical discussions dealing with the heart, intended primarily for the busy general practitioner of medicine, who usually is accorded little consideration by medical authors. It was on this account that all the discussions were brief; each could be read in less than ten minutes, and this brevity has been maintained."

The Reviewer agrees that the general practitioner can find much interesting information in this little book. W. J.

STRANGE MALADY. The Story of Allergy. By WARREN T. VAUGHAN, M.D. Pp. 268; 38 illustrations. New York: Doubleday, Doran & Company, 1941. Price, $3.00.

THIS is the second in a series of non-technical books sponsored by the American Association for the Advancement of Science and addressed to the intelligent lay public. The Association is to be doubly congratulated, both on the choice of subject and the choice of the author. It is a conservative estimate that a third of all people experience allergy in themselves or their relatives. The recognition and especially the treatment of the manifestations of allergy therefore depend to a considerable extent upon the intelligent coöperation of the patient and his family. The author brings to his task not only a profound knowledge of his subject but the happy faculty to present in a vivid, fascinating and lucid manner the complexities of theory that clutter this field. Thus the abstractions of the side-chain theory, of immunity, and of anaphylaxis are made clear and understandable to everyone. Physicians as well as laymen will find this book valuable as well as interesting reading. R. K.

HANDBOOK OF COMMUNICABLE DISEASES. By FRANKLIN H. TOP, A.B., M.D., M.P.H., Director, Division of Communicable Diseases and Epidemiology, Herman Kiefer Hospital and Detroit Department of Health, etc., and Collaborators. Pp. 682; 73 illustrations and 10 color plates. St. Louis: The C. V. Mosby Company, 1941. Price, $7.50.

THE purpose of this book is to furnish practical information for all persons whose professional duties necessitate contact with certain communicable diseases. Thus it will prove to be a handy text not only for students, practitioners of medicine, and nurses, but also for lay personnel in health departments. The first section deals with general considerations applicable to communicable diseases, with chapters on infection and immunity, epidemiology, regulations governing control of communicable diseases, specific prevention, serum and serum reactions, management of communicable diseases in the home, in the hospital. The second section is devoted to the individual diseases, classified by common portal of entry. The third section includes tables epitomizing the contagious disease experience in the Herman Kiefer Hospital over a 10-year period, including complications and diagnostic problems; a table listing the case, contact, and dwelling status of reportable infectious diseases under the laws of Detroit; a glossary of nearly 400 medical terms. While not sufficiently detailed to serve as a general work of reference, the book is entirely adequate for prac-

tical purposes. The general level of excellence is of a high order. Features worthy of separate mention are: the general discussions of control and prophylaxis; the chapters on management of communicable diseases in the home and in hospital (written by nurses); the tables on communicable disease statistics from the Herman Kiefer Hospital; the many excellent illustrations. In a few instances the description of specific prophylaxis is not sufficiently detailed. The book should find a wide appeal. R. K.

NEW BOOKS.

William Henry Welch and the Heroic Age of American Medicine. By SIMON FLEXNER, and JAMES THOMAS FLEXNER. Pp. 539; illustrated. New York: The Viking Press, 1941. Price, $3.75.

A Manual of the *Treatment of Fractures*. By JOHN A. CALDWELL, M.D., Professor of Clinical Surgery, College of Medicine, University of Cincinnati; Director of the Fracture Service, Cincinnati General Hospital, Cincinnati. Pp. 150; 76 illustrations. Baltimore: Charles C Thomas, 1941. Price, $3.50.

Hippocratic Medicine. Its Spirit and Method. By WILLIAM ARTHUR HEIDEL. Pp. 149. New York: Columbia University Press, 1941. Price, $2.00.

Lectures on *War Neuroses*. By T. A. Ross, M.D., F.R.C.P. Pp. 116. Baltimore: The Williams & Wilkins Company, 1941. Price, $2.00.

Immunization to Typhoid Fever. (The American Journal of Hygiene Monographic Series, No. 17, September, 1941.) From the Research Laboratories of the Army Medical School, Washington, D. C. Supported by the De Lamar Fund of The Johns Hopkins University. Pp. 276; illustrated. Baltimore: The Johns Hopkins Press, 1941. Price, $2.50.

An X-Ray Atlas of Silicosis. By ARTHUR J. AMOR, M.D. (Lond.), M.Sc. (Wales), Honorary Physician, Clydach Memorial Hospital; Medical Officer, Mond Nickel Company, Ltd., Swansea. With Translation of the Legends into French by ROBERT E. HORNE, M.A. (Wales), Medical Secretary, Mond Nickel Company, Ltd., Swansea. With a Foreword by SIR WILSON JAMESON, M.A., M.D. (Aberdeen), F.R.C.P., of the Middle Temple, Barrister-at-Law; Dean of the London School of Hygiene and Tropical Medicine. Pp. 206; 6 text illustrations and 72 plates. Baltimore: The Williams & Wilkins Company, 1941. Price, $8.00.

Shock Treatment in Psychiatry: A Manual. By LUCIE JESSNER, M.D., PH.D., Resident Psychiatrist, Baldpate, Georgetown, Mass.; Graduate Assistant in Psychiatry, Massachusetts General Hospital; Assistant in Psychiatry, Beth Israel Hospital, Boston, and V. GERARD RYAN, M.D., Associate Psychiatrist, Elmcrest Manor, Portland, Conn.; Assistant in Psychiatry, Harvard Medical School. Introduction by HARRY C. SOLOMON, M.D., Clinical Professor of Psychiatry, Harvard Medical School; Chief of Therapeutic Research, Boston Psychopathic Hospital. Pp. 149. New York: Grune & Stratton, Inc., 1941. Price, $3.50.

Pathology of the Oral Cavity. By LESTER RICHARD CAHN, D.D.S., Associate Professor of Dentistry (Oral Pathology), Columbia University, etc. Pp. 240; 165 illustrations. Baltimore: The Williams & Wilkins Company, 1941. Price, $5.50.

The Premature Infant. Its Medical and Nursing Care. By JULIUS H. HESS, M.D., Professor and Head of the Department of Pediatrics, University of Illinois College of Medicine; Attending Pediatrician, Illinois Research and Educational Hospital, Cook County and Michael Reese Hospitals, and EVELYN C. LUNDEEN, R.N., Supervisor, Premature Infant Station, Sarah Morris Hospital, Chicago. Pp. 309; 63 illustrations. Philadelphia: J. B. Lippincott Company, 1941. Price, $3.50.

Journal of Technical Methods and Bulletin of the International Association of Medical Museums, No. XXI, October, 1941. Editorial Board: ROBERT A. MOORE, Editor, J. LUDWIG ASCHOFF, J. EARLE ASH, WILLIAM BOYD, VIRGIL H. CORNELL, TRACY B. MALLORY, MATTHEW J. STEWART, CARL V. WELLER. Pp. 88; illustrated. Toronto: International Association of Medical Museums, 1941.

The Symposium in this number on the use of x-rays in pathology is of especial interest. The single article on congenital cardiac malformations reminds one of the prominent part that this journal has taken in the past in presenting the English-speaking literature on this subject. It is to be hoped that with Dr. Abbott's death this feature will not be lost. E. K.

Epilepsy and Cerebral Localization. A Study of the Mechanism Treatment and Prevention of Epileptic Seizures. By WILDER PENFIELD, LITT. B., M.D. (Johns Hopkins), D.Sc. (Oxon., Princeton), Professor of Neurology and Neurosurgery, McGill University; Director of the Montreal Neurological Institute, and THEODORE C. ERICKSON, M.A., M.Sc., M.D. (Minnesota), PH.D. (McGill), Associate Professor of Surgery, University of Wisconsin. Chapter XII by HERBERT H. JASPER; Chapter XX by M. R. HARROWER-ERICKSON. Pp. 623; 163 illustrations. Springfield, Ill.: Charles C Thomas, 1941. Price, $8.00.

Teaching Preventive Medicine to Medical Students. With Special Reference to the Use of Health Department Facilities. By HUGH R. LEAVELL M.D., DR. P. H. Pp. 77. New York: The Commonwealth Fund, 1941.

Strange Malady. The Story of Allergy. By WARREN T. VAUGHAN, M.D. Pp. 268; 38 illustrations. New York: Doubleday, Doran & Company, 1941. Price, $3.00. (Review, p. 884.)

NEW EDITIONS.

Practical Methods in Biochemistry. By FREDERICK C. KOCH, Frank P. Hixon Distinguished Service Professor of Biochemistry, University of Chicago. Pp. 314; illustrated. Third Edition, revised. Baltimore: The Williams & Wilkins Company, 1941. Price $2.25

In this edition new methods for uric, lactic and pyruvic acids have been added. Except for typographical corrections, no other changes have been made in this excellent laboratory manual. D. W.

Diseases of the Blood and Atlas of Hematology. With Clinical and Hematologic Descriptions of the Blood Diseases Including a Section on Technic and Terminology. By ROY R. KRACKE, M.D., Professor of Bacteriology, Pathology and Laboratory Diagnosis, Emory University School of Medicine; Pathologist to the Emory University Hospital, etc. Pp. 692; 46 text illustrations and 54 color plates. Second Edition, thoroughly revised, reset and enlarged. Philadelphia: J. B. Lippincott Company, 1941. Price, $15.00.

The Modern Treatment of Syphilis. By JOSEPH EARLE MOORE, M.D., Associate Professor of Medicine, The Johns Hopkins University; Physician-in-charge Syphilis Division of the Medical Clinic and Visiting Physician, The Johns Hopkins Hospital, etc. With the Collaboration of JAROLD E. KEMP, M.D., Associate in Venereal Diseases, The Johns Hopkins University; HARRY EAGLE, M.D., Passed Assistant Surgeon, United States Public Health Service and Lecturer in Medicine, The Johns Hopkins University; PAUL PADGET, M.D., Associate in Medicine, The Johns Hopkins University, and MARY STEWART GOODWIN, M.D., Instructor in Pediatrics, The Johns Hopkins University. Pp. 674; 98 illustrations. Second Edition. Springfield, Ill.: Charles C Thomas, 1941. Price, $7.00.

PROGRESS
OF
MEDICAL SCIENCE

PEDIATRICS.

UNDER THE CHARGE OF
ALVIN E. SIEGEL, M.D.,
MACON, GEORGIA.

POLIOMYELITIS—A SURVEY OF RECENT CONTRIBUTIONS.

In this department in January, 1934, the then recent literature of poliomyelitis was reviewed. Since that time there have been one or more epidemics of this disease each summer with result that many contributions to the subject have appeared. These have included clinical and case reports, statistical studies, as well as reports of investigative study. The sum total of this literature is a mountainous mass of articles, but boiled down to a concrete basis very little has been added to our knowledge of this disease.

Our interest in this disease has been reawakened this year by the epidemic especially in the southeastern section of this country, which began in the early summer in several cities of Florida and insidiously spread to neighboring states. A great deal of interest has been developed from the epidemiologic viewpoint because of the very large concentrations of young men in the military service in the South, as well as because of the fact that these soldiers have been further concentrated in certain areas on maneuvers to which have been added thousands of troops from posts in the North. It is not possible so early to determine to what extent the young men in the army have contracted the disease and to what degree extension of the areas of epidemic has occurred by the return of military units from epidemic areas to their home stations where epidemic had not prevailed.

According to Sabin[28] poliomyelitis is an acute infectious disease that is caused by a filtrable virus with sufficiently distinct properties to permit its identification with as much certainty as is possible to identify any of the well-known bacteria. The etiologic agent of poliomyelitis is known to be one of the smallest filtrable viruses. It can pass through the finest filters and membranes, which retain not only the bacteria but most viruses as well. It can be sedimented only by prolonged centrifugation on powerful ultracentrifuges and it is known that the individual virus particle is large enough to accommodate only a few molecules the

size of hemoglobin. Of all the viruses known to attack the human nervous system, the virus of poliomyelitis has the most limited host range. The virus, as contained in human tissue or excreta, has previously been found to be pathogenic only for certain monkeys and chimpanzees but not for mice, guinea pigs or rabbits but reports of new hosts are included later in this review. Poliomyelitis virus possesses highly specialized tissue affinities. From numerous studies it has been found that poliomyelitis virus never multiplies in cells other than the neurons of susceptible hosts. Sabin summarized the course of events in human poliomyelitis on the basis of present knowledge as follows: "It seems to me that one may be permitted to visualize the virus entering the human body by way of the mouth and establishing itself throughout the alimentary tract, where it proceeds to multiply. While further work is necessary to indicate to what extent various levels of the alimentary tract are affected, there is already more than suggestive evidence that the walls of the pharynx and small intestine are among the chief sites of virus multiplication, and the feces, the chief means of virus elimination. Whether the virus multiplies in the nerve cells, which are contained in the walls of the alimentary tract, or in the non-nervous tissues remains unknown. However, whatever the type of cells affected in the alimentary tract, the virus may be visualized as beginning its invasion of the central nervous system essentially along two pathways: one leading into the medulla by way of the cranial nerves that supply the upper part of the tract or by way of the parasympathetic system along the vagus from the lower part, and the other leading into the spinal cord either along the visceral afferent fibers through the spinal ganglions or along the efferent fibers from the intestine by way of the abdominal sympathetic ganglions. If the greater attack and spread of the virus is along the first pathway one may expect a syndrome in which bulbar signs are primary, while if the second pathway is more predominatingly affected the primary paralysis would be in the extremities. That these pathways perhaps may be used simultaneously, the outcome depending on the route by which the virus arrives in the central nervous system first, is suggested by the fact that in a purely bulbar case the virus has been found in the pharynx, ileum, colon and abdominal sympathetic ganglions. The inapparent or abortive type of case would be either one in which virus multiplication is limited to the alimentary tract or one in which the virus also invades the central nervous system but fails to destroy a sufficient number of cells to give rise to clinical signs. This view is based in part on the recent finding in monkeys inoculated with human virus that an equilibrium may be reached between the host and the virus before sufficient neurons have been destroyed to interfere with apparently normal function. Similarly, in the non-paralytic type of poliomyelitis, the virus may be visualized as multiplying in the alimentary tract and invading the central nervous system, but the activity of the virus is suppressed before a sufficient number of neurons to give rise to paralysis has been destroyed. That the virus may continue to multiply in the alimentary tract long after the disappearance of clinical signs of the disease is evident from the fact that in some instances the virus has been found excreted in the stools for weeks and months after clinical recovery. . . . ."

The foregoing theory of the means or portal of entry of the virus of

this disease into human body exemplifies the trend of thought on the subject. Until recently the nasopharynx was thought to be the portal of entry, and different chemical agents were recommended for spraying into the nasopharynx as a means of prevention against the disease. Commenting on the port of entry as a result of very recent contributions, an Editorial[7b] observes that many factors point to the alimentary tract as a locus of virus proliferation. In many instances the material suggests that the spinal cord is the primary site of the invasion of the central nervous system. It has not been possible to determine the exact pathway of the virus from the intestine to the central nervous system except that the vagus nerve has been strongly suspected. There has also been evidence that the nerves supplying the pharynx were the path of entry. There is evidence that there may be many ports of entry and that the olfactory route is of little importance in man.

Zahorsky[37] stated that the theory that the nasal route is the portal of entry of the virus of poliomyelitis was not convincing. He thought that the gastro-intestinal tract might be the portal of entry, and that the contamination of fresh fruits and other foods, milk products and water by flies and their excreta was worthy of consideration. He pointed out that while across the river from St. Louis poliomyelitis was endemic for a number of years, there were only isolated cases of the disease in St. Louis. This reference is of interest as Zahorsky suggested an alimentary portal of entry at a time when it seemed definitely established that the nasopharynx and the olfactory system were culpable. The general opinion at this time is strongly in favor of the entrance of the disease into humans by way of the alimentary tract although there are some who still have not been convinced that the olfactory theory of entrance has been disproved. Sabin and Ward[29] revalued the prevailing hypotheses of the disease in human beings. The absence of demonstrable virus in the olfactory bulbs and anterior perforated substance indicated that in man the olfactory pathway need not be affected. The absence of infective virus in the nasal mucosa suggested that this is not the site of virus multiplication and dissemination. The virus was not present in the salivary glands, which indicated that the saliva is not an elimination route. The positive results with the tonsils and pharyngeal mucosa were probably due to the pharyngeal tissue rather than to the tonsils. In the alimentary tract the virus was present not only in the content but also in the washed walls of various parts of the tract, including the pharynx, the ileum and occasionally the colon. They thought that infection of the alimentary tract was the result neither of primary localization nor of portal of entry. The distribution in the central nervous system was found to be limited to certain areas, and it was not as indiscriminately disseminated as viruses such as equine encephalomyelitis which can invade through the blood-vessels or those such as rabies which are capable of extensive centrifugal spread. The pattern of virus distribution in human poliomyelitis, according to this study, pointed to almost the entire alimentary tract as the primary site of attack by the virus and no support was found for the concepts involving either the olfactory or the respiratory systems. The distribution also militated against the cutaneous route although it may be conceivable that occasional infection through the broken skin may be possible.

Swan,[33] examining the central nervous system of 8 patients with poliomyelitis by routine neurohistologic methods, found that 4 of these cases showed an absence of changes in the olfactory bulbs. He believed that infection by the virus of poliomyelitis by the olfactory route is either less common than has been thought or it is less fatal. Because the most intense changes were seen in the tissues forming the floor of the fourth ventricle, which extends through the whole length of the brain stem and below is continuous with anterior intersegmental tract of the spinal cord, this median longitudinal bundle must be considered as an important path for the spread of the pathologic process.

Studies of the stools of individuals suffering from poliomyelitis have revealed the presence of the virus. Toomey[34b] has demonstrated that the virus of anterior poliomyelitis can be recovered from the stools of human beings ill with the disease. It has also been proved that the virus is present in sewage in areas where the disease is prevalent. Toomey is of the opinion that the virus of poliomyelitis enters the human system by way of the gastro-intestinal tract and that there is no support for the theory of nasal passage as even an occasional port of entry of the disease.

Kramer[33] reported a study from children in a separate wing of a small institution comprising 20 infants and children of preschool age. Of this group, 5 contracted poliomyelitis with fatal results in 1 child and non-paralytic results in 4 children. In 3 additional children there was evidence of fever of from 24 to 48 hours' duration. The virus of poliomyelitis was recovered from the stools of 3 of the 12 remaining healthy children, from contacts of children having the disease and from 2 of the 3 children who had fever but for whom the diagnosis of poliomyelitis was not established. In all, 10 of the 20 children harbored the virus of poliomyelitis sometime during the 30-day period of observation.

While the presence of the virus of the disease in the stools seems indisputable, that another factor is present in the stools of patients ill with poliomyelitis is suggested by Toomey and Takacs.[35c] They commented that stasis of the intestine occurs frequently in patients with poliomyelitis. The toxic factor present in their stools may be due to the increase of bacterial virulence of resident organisms. The effect of the bacteria present in the stool did not seem important, since a sterile fecal eluate was just as toxic as the whole stool. These experiments suggested to the authors that convalescent poliomyelitis serum may have the ability to neutralize not only poliomyelitis virus but also a toxic factor present in the gastro-intestinal tract. That this is not improbable was seen from the fact that the agglutinin titer against enteric organisms rose as the experimental animal with poliomyelitis became better.

The virus has been demonstrated in the stools by a number of observers besides those quoted. The presence of the additional toxic factor, as suggested by the work of Toomey and Takacs, has not caused the publication of confirmatory or contradictory opinions some 6 months after the appearance of their paper. It may be that such will appear after sufficient time has elapsed. Little or no sponsorship of the nasal or olfactory port of entry is found in the literature of the past 4 years. Therefore, until disproved by new evidence, the alimentary tract should be accepted as the portal of entry.

Granting that this theory of alimentary transmission is correct, the question naturally arises as to how the infectious material reaches the individual. Inoculation might occur by partaking of foods or fluids that have been contaminated with the infectious material in a manner similar to that in which typhoid fever transmission occurs. Ellsworth[9] cited the report of Lovett and Richardson who in 1911 reported that, in 419 cases of infantile paralysis observed by them from 1908 to 1910, 105 patients (slightly more than 25%) had swum, waded or paddled in more or less contaminated waters just prior to the attack. Precedent accidents, overexertion and falls were reported in another group of cases. Of these factors overexertion definitely lowers the body resistance and it is conceivable that this may predispose to infection. Not only overexertion but prolonged periods of bathing or swimming, in which the body gets severely chilled, may act in a similar manner.

To Paul and Trask[25] it has become increasingly apparent during the past 2 years that the virus of poliomyelitis may be readily isolated from the stools of patients with poliomyelitis. This is true not only in cases of the paralytic type but the virus has also been demonstrated in the stools of patients with the abortive type of the disease. From their study these authors commented that newer methods of testing human feces and sewage have been of assistance in detecting the existence of the virus of poliomyelitis throughout an infected community during epidemic times. River and harbor water and large volumes of sewage were tested in three urban epidemics. The virus of poliomyelitis was detected in two sewers both of which were in the vicinity of hospitals, and from these tests it was learned that not only can the virus content of sewage be large but the virus may be transported for short distances at least in flowing sewage. They believe that poliomyelitis is probably transmitted by a number of different channels. Among these is the direct or indirect factor of close intimate contact between infected persons, illustrated by the type of interfamilial contact in which juvenile members of a family usually share. Many of these infected persons may be convalescent carriers in the first few weeks of their recovery from either a diagnosed or a missed case, and some of the exposed persons may become transient "healthy" carriers. There may be also a variety of other channels, in which contaminated foods, milk and water or possibly insects, mammals or birds, play a part. Watercourses and particularly watercourses polluted with sewage may be one of the channels of transmission.

The fact that difficulties arise in the transmission of poliomyelitis in man to experimental animals has given rise to the general belief that other animals than man and certain species of monkeys are not infectable with the disease. Frauchiger and Hoffman[12] attempted the transmission of the disease to cattle. Poliomyelitis material from human subjects was inoculated into 3 heifers. Large doses were given by the intranasal, intraperitoneal and intraspinal routes. Except for increase in the temperature at the onset, general symptoms, such as gastrointestinal disturbances, nasal discharges and sweating, were absent. There were no signs of meningeal irritation. However, after 2 or 3 days signs of paralysis appeared in the extremities, particularly the posterior. The paralytic symptoms increased until the seventh day, but there was never complete flaccid paralysis. This was a very interesting study

and Frauchiger and Messerli[13] were interested in paralysis in other domestic animals. They described observations on 2 hogs which presented symptoms that greatly resembled those of acute anterior poliomyelitis. On this basis such a diagnosis was made and both animals were killed. Study of the spinal cords revealed histologic aspects resembling those of acute poliomyelitis in human subjects. Material from the spinal cords of these animals was introduced into two hogs and into one steer. Because of inadequate technique, nothing could be learned from the inoculation test on the hogs; but the steer, in which a formerly devised technique was employed for the inoculation, developed fever of a "dromedary" (camel) type. Other symptoms included signs of fatigue, stiffness of the neck and an ataxic walk. From the seventh day after the inoculation the symptoms commenced to subside and a few days later the animal seemed normal again. The animal was killed 14 days after the inoculation. The histologic findings of the central nervous system were those of a lymphocytic meningo-encephalomyelitis rather than those characteristic for poliomyelitis. However, it was possible that had the animal been killed during the acute stage of the disease lesions more typical of poliomyelitis might have been found. Because of Spielmeyer's demonstration that similar histologic aspects do not necessarily indicate the same disease, etiology or causal agent, the authors did not definitely conclude that the described paralytic symptoms in hogs were identical with human poliomyelitis, but they did believe that this could be the case.

In Burnet's[4] epidemiologic experiments the most important results indicated that infection did not produce poliomyelitis antibody, that certain children with initial antibody nevertheless suffered from paralytic infection and that cynomolgus monkeys could be infected with the local virus by ingestion or by pharyngeal swabbing. As regards age incidence, he was led to the conclusion that an important change in the character of the disease had taken place in the last 30 or 40 years. It would seem that in the earlier period poliomyelitis was essentially an adult infection with incidental involvement of infants, while now epidemic poliomyelitis is a disease of childhood, and spread by child contact.

Aycock[2] made observation on 1004 patients or photographs of patients with various crippling conditions to test the clinical impression that paralytic poliomyelitis exhibits a tendency to selective occurrence in certain types of individuals. The significant proportion of correct readings obtained confirmed this impression and verified Draper's concept of a "poliomyelitis type," as well as anthropometric studies indicating a difference in bodily measurements from the normal. There was additional evidence to support the epidemiologic observation that the frank disease tends to occur in but a limited and selected fraction of those exposed. These individuals are possessed of an autarceologic susceptibility as shown by the disease to familial aggregation and that the character in question is a physiologic difference of an endocrine nature, which, although to a large degree subclinical, manifests itself in the clinically discernible characteristics of the individuals. This occurs in a slight degree in younger girls, moderately in younger boys and older girls, and to a still greater degree in boys 10 years of age and older.

In Dauer's[5] study of 231 cases of poliomyelitis in children under 13 years of age, from 90% to 95% had some paralysis, and the remainder had, besides the clinical symptoms, an increase of cells in the spinal fluid. From the standpoint of geographic location, there was evidence that there may be some form of direct or indirect contact between patients or a common source of infection or both. In 2 instances the disease appeared in 2 members of the same family; in 2 instances it appeared in 2 persons in the same institution; in 2 instances it developed in different families in the same dwelling, and in 3 instances the disease was reported from adjoining houses.

Helms and Willcocks[17] in their report commented on the meteorologic factors in relation to the increased incidence of cases. Commencing in spring, the cases attained a maximum in midsummer and then gradually declined. A somewhat similar correlation was suggested in the incidence of seasonal rainfall in northern parts of New South Wales. In the metropolitan area, during early weeks of the epidemic and while the weekly incidence was increasing, severe paralysis developed in a greater proportion of patients than during the later stages of the epidemic. In rural districts this tendency may have been masked by the fact that a relatively small population is spread over a large area. The number of deaths was greater in the rural districts at the beginning and at the height of the epidemic than in the later stages. In the metropolitan area there appeared to be a tendency for the age group up to 4 years to be proportionately larger and the group more than 15 years of age to be proportionately smaller at the beginning of the epidemic than in its later stages. This tendency may have been masked in the rural districts for the same reason that the severity of the paralysis was masked. Among other factors they discussed the relation of tonsil and adenoid operations in the occurrence of poliomyelitis. Of 711 patients 7 were known to have had a tonsillectomy and adenoidectomy from 7 to 22 days prior to the onset of the illness. Bulbar and spinal paralysis developed in 3 of these 7 patients, 2 had paralysis of the palate, 1 had spinal paralysis with no evidence of bulbar involvement and 1 patient had the non-paralytic form of the disease. There were no deaths.

Eley and Flake[8] stated that the fact that a bulbar form of acute anterior poliomyelitis had been reported as following recent tonsillectomy and adenoidectomy has suggested that this procedure may in some manner influence the entrance of the virus. They studied 418 consecutive patients with poliomyelitis. There were 287 patients with spinal poliomyelitis, and in 8 of these the disease occurred within 30 days after the removal of the tonsils and adenoids. In only 3 of these did the disease develop within 7 to 18 days after the operation. Of 131 patients with bulbar poliomyelitis, 17 had this operation performed within 20 days prior to the onset of their illness. In 15 of these 17 patients the disease developed within the incubation period of from 17 to 18 days.

Koskoff, Amshel and Lebeau[22] reported 2 cases of bulbar poliomyelitis following tonsillectomy and adenoidectomy. They stated that, although their patients may have developed the disease without the operation, this did not seem likely. The patients were both well preoperatively. The onset of the disease falls well within the experimental incubation

period. It is suggested that the trauma of the operation opens avenues of infection along the nerves of the pharyngeal region to the medullary nuclei. This is, of course, the avenue of infection that for some years was supposed to be the means of entrance of the virus of poliomyelitis into humans. As has been discussed above the alimentary tract has come now to be considered the portal of entry.

Fischer, Stillerman and Marks[11] in a very recent publication commented on the increasing interest in the relation of tonsillectomy and adenoidectomy to poliomyelitis. Most of these cases following operation have shown the bulbar form. They studied the records of an epidemic that occurred in Toronto. These records were kept in an excellent manner with especial reference to the history of previous tonsillectomy and the date of the operation. The first part of this study entailed the collection of data on 507 patients with poliomyelitis who were from 3 to 12 years of age at the time of the epidemic. The patients were classified as tonsillectomized and non-tonsillectomized, and the two groups were analyzed according to the month of occurrence, the age at the time of onset, the type of and the mortality from poliomyelitis and the relation of the length of the interval between tonsillectomy and onset of the disease to the type of poliomyelitis. It was found that poliomyelitis took the bulbar form in the largest proportion of those patients in whom the disease developed within 1 month after tonsillectomy. Also the percentage of cases of the bulbar form among the children with poliomyelitis who had a history of tonsillectomy, regardless of when performed, was over twice as high as that in the non-tonsillectomized children with the disease. This difference was in conformity with the statistics on types of poliomyelitis in the series reported by Eley and Flake (*loc. cit.*). In the second part of this study, with the coöperation of public health and school officials, a large sample of the school population was surveyed to obtain information on the general incidence of tonsillectomy. It was shown what was the actual number of school children in the sample who had been tonsillectomized at any time and the date of the operation in each case. From these data it was possible to compute the incidence of poliomyelitis during this epidemic among recently tonsillectomized children. This part of their study showed a higher incidence of poliomyelitis in the recently tonsillectomized group than among other children, but since the total number of cases of poliomyelitis in the tonsillectomized group was only 8, a number too small to give a statistically significant difference, the result by itself cannot be considered conclusive. The frequent reports in late years of the occurrence of poliomyelitis in recently tonsillectomized children does give some confidence in the significance of the reported study. They pointed out that on epidemiologic grounds alone there would not be expected a higher incidence of poliomyelitis among recently tonsillectomized children than among other children, because other things being equal, there is no reason to expect any difference between the degrees of exposure of the two groups. If anything, the recently tonsillectomized children seemed to be less exposed, so far as they were isolated from other children during the period of operation and convalescence. The higher incidence of poliomyelitis among the recently tonsillectomized children suggested a lowered resistance to the disease. The mechanism of this lowered resistance

was not altogether clear, but probably it was related either to the portal of entry of the disease or to the avenue of its spread. It was thought that traumatized adenoid and tonsillar tissue may afford the portal of entry for the virus in some cases. One suggestion was that the tonsils and adenoids serve as a barrier. Their removal eliminated this barrier with the result that the virus often gained a foothold.

Numerous recent studies[7a] on the immunologic reactions of the poliomyelitis virus have resulted in a considerable extension of knowledge of its behavior. Successful adaptation of human poliomyelitis virus to the cotton rat and white mouse is one of the most promising advances in practical research technique of the present decade. Earlier, monkeys were the only susceptible animal species until Armstrong[1a] reported the transference of rhesus poliomyelitis virus to the eastern cotton rat. The disease thus produced in cotton rats was characterized by flaccid paralysis of the extremities associated with degenerative lesions of the anterior horn cells. These lesions were apparently identical with those usually observed in human poliomyelitis and in experimental poliomyelitis in monkeys. The identity of the rodent infection with rhesus poliomyelitis was further indicated by the fact that the rodent infection would produce typical poliomyelitis symptoms and lesions on return inoculation into monkeys. It was later reported by Armstrong[1b] that the rhesus virus, once serially adapted to the cotton rat, could be successfully transferred to white mice. Proof of this transference was incomplete because it was found that the mouse virus was non-pathogenic on return inoculation into monkeys. This loss of pathogenicity suggested that the human poliomyelitis virus might have been lost and the observed symptoms in mice might have been due to some unknown neurotropic environmental virus. In order to complete the proof of the successful transference of the human virus to mice, Jungeblut and Sanders[20a,b] studied the antigenic properties of the passage virus directly isolated from the mouse brain and cord or after propagation in mouse tissue culture. Confirming earlier investigators, they found that on serial inoculation into the cotton rat the human virus rapidly lost its pathogenicity for monkeys. Simultaneously with this attenuation for the rhesus monkey there was an increase in rodent pathogenicity. Still further increases were noted in the mouse until a "fixed" virus was eventually obtained, which was wholly non-pathogenic for monkeys. This fixed virus could be successfully transmitted and adapted to mice, by preliminary serial passage through monkeys and cotton rats. The most encouraging results have been the claimed successful immunization of monkeys by the repeated subcutaneous injections with living mouse virus. This specifically attenuated viable murine vaccine is readily grown in artificial tissue cultures. While the immunity conferred by this viable vaccine is not absolute, it is sufficiently effective to stimulate the hope that better results will follow further clinical research. If this vaccine does ultimately prove effective, Armstrong's original observations may become one of the major discoveries of modern research medicine.

Haas and Armstrong,[16] in the mouse protection test, using 83 human serums and the Lansing strain of poliomyelitis virus, found that serum antibodies capable of neutralizing the Lansing strain of poliomyelitis virus were widely prevalent especially among older individuals. The

results secured with mice appeared to be more trustworthy than those usually secured with monkeys, as mice are more uniformly susceptible. The results agreed with those obtained with human serum in monkeys. These considerations should render it possible to follow serum immunity in population groups from different localities and from infancy to adulthood and thus ultimately clarify many epidemiologic questions awaiting solution.

Barber[3] reported 4 cases of anterior poliomyelitis occurring on the same day in a boys' school. Since no other cases developed in this school, a close analysis was made of the daily habits and lives of these boys. Cases did appear later in the village and 2 of them occurred in the family of one of the maids employed by and living in the same house of the school in which the 4 boys lived. These cases were attributed to the maid carrying the infection home. As she was in the house for the rest of the term and no further cases occurred there, and as one attempt to infect a monkey from her proved negative, there was no actual evidence that she was a carrier. The house in which the 4 boys lived had separate feeding arrangements and a separate domestic staff from the rest of the school. Only 2 of the boys slept in the same dormitory. The other 2 were in different dormitories, and each was unconnected with the others in any activity. In the house-room all 4 boys were in different corners and 1 of them was present only occasionally as prefect. In the school all 4 were well scattered, being in different classes and other categories. The only common factors appeared to be the same feeding arrangements and contact with the domestic staff. The only connection with food was that strawberries, obtained from 2 farms, 1 of which had been affected, were served only in the house where the affected boys lived. The simultaneous onset certainly resembled a food-poisoning outbreak rather than an epidemic spread by droplet infection.

Until now there has been no definite or generally accepted idea of the transmission of poliomyelitis. It would seem that the theory of the alimentary tract as the portal of entry would furnish the best explanation of the rather small incidence of the disease in relation to the population even in epidemics. It has been thought that many escape the infection because of having developed immunity. Such has been the idea in reference to adults. However, although frequent small infections with the causative factor may give rise to the immunity that most adults seem to have, sometimes even a definite attack may not give rise to complete immunity, so that second attacks have been recorded. Toomey[34a] recorded the eighteenth case of a second attack of poliomyelitis. This case was similar to the cases reported by others but it was not felt that it was a bona fide second attack. The objection was based on the theory that a person who has had poliomyelitis is a prey not so much to another attack of the same disease as to other diseases, such as influenza, measles and scarlet fever, which involve the few remaining peripheral nerve fibers and their cells of origin. Toomey felt that the subsequent paralysis in his case were the result of influenza affecting the remaining nerves and cells. It could have been toxic neuronitis in a person who had an old poliomyelitis. In the presence of any acute infection, such as influenza, bronchopneumonia, measles, coryza due to the virus of the common cold or scarlet fever, or in the

presence of an injury, it is right to question the diagnosis of a second attack of poliomyelitis and to insist that first toxic neuronitis be ruled out. If acute infections do not aggravate the clinical condition of an old poliomyelitis, as has been shown experimentally, few of the reported cases in the literature can qualify as actual instances of a second attack of poliomyelitis.

Faber[10a] visualized poliomyelitis as a disease due to a progressive invasion of the nervous tissue by a virus which is not only neurotopic but strikingly selective within the nervous tissues. It is capable of producing only transient lesions such as hyperemia and cell infiltration in certain parts of the central nervous system as the upper part of the brain stem, destructive lesions or cell degeneration in only a few areas such as anterior horn cells and motor cells of the medulla and slight lesions or none at all in other areas such as the cerebral cortex and cerebellum. In many instances the virus plants itself for a brief time only as it may invade the olfactory bulbs and sometimes the brain stem and then die out without reaching the vulnerable lower motor neurons. These cases are the abortive and non-paralytic types of poliomyelitis. The symptom complex of the disease shows an order of progression corresponding with the advancing distribution of lesions and may be divided into three phases. The first phase is manifested by fever alone and the lesions are confined to the olfactory bulb. The second phase consists of a symptom complex in which more or less generalized effective, sensory and autonomic phenomena are conspicuous, and the lesions consist mainly of basal encephalitis without involvement of the cord. During the third phase there occur two kinds of symptoms. The first kind are autonomic and sensory phenomena arising in the lateral and posterior horns and dorsal root ganglions. The second kind are localized phenomena arising in the anterior horns.

Toomey[34c] believes that poliomyelitis virus has but a slight affinity for the exposed axons or peripheral medullated nerves, and that it may be that its spread to the central nervous system is stopped by the myelin contained in the sheath which covers the gray fibers. This would explain why there is not in every case of poliomyelitis involvement of the vagus nerve, which is actually a bundle of well-medullated connector fibers. Although these gray fibers might be partly or completely involved in every case of poliomyelitis, few symptoms of this involvement other than constipation would become manifest clinically. Experiments supported the belief that ordinarily virus of poliomyelitis does not spread along well-myelinated nerves of healthy monkeys but that virus will spread along nerves of vitamin D-deficient monkeys if the virus is simply placed in contact with the postganglionic fibers. It is presumed that in the latter animals the preganglionic medullated nerve fibers going to the adrenal gland are made deficient and consequently more susceptible.

Studying the spinal fluid in an outbreak of poliomyelitis, Drury and Sladden[6] found that detailed and routine analysis of the spinal fluid was a very valuable procedure in the detection and diagnosis of poliomyelitis and also in polioencephalitis. Where a completely normal spinal fluid is found the diagnosis of the disease is negative. Slight pleocytosis is a constant feature of the disease and there are always a few neutrophils present in the early stages. Usually there is slight

protein increase and this becomes more marked with the progress of the disease. Globulin is always found, although it may be only a trace. The Lange gold curve often affords useful corroborative evidence.

Hunt[18] stated that the changes in the spinal fluid varied with the stage of the disease and with the reaction of the meninges. In the systemic phase the fluid usually increased in amount, the cell count was from 30 to 100 per c.mm., and the aggregate of cells was made up entirely of neutrophils or of both leukocytes and lymphocytes. The globulin was increased in some cases and not in others. In the stages of paralysis the fluid was still increased in amount, the number of cells varied from 18 to 2000 or more and from 80% to 90% of the cells were lymphocytes. In general, the highest cell counts were shown by the patients showing symptoms of severe meningeal irritation. In the fatal cases the cell counts of the spinal fluid were invariably high, averaging above 200 cells.

Meyer[24a] studied the leukocytic formula in 57 cases of poliomyelitis and also in a varying number of cases of other conditions which might be confused with it. He found that in cases of poliomyelitis during the period of invasion and the first days of paralysis there is a slight leukocytosis, with a slight increase in neutrophils. This gives way little by little to a slight lymphocytosis, with the occasional appearance of rare plasmocytes and a monocyte count of 9% or more. The eosinophils and basophils tend to disappear. After the establishment of the paralysis, the leukocytic formula returns to normal, or there may be a slight leukopenia. The only other condition which might be confusing from the standpoint of the blood picture was acrodynia, but the gradual onset makes the clinical differentiation easy.

Spector and Hobart[32] commented that the most characteristic finding in their series was the resistance of the patient against flexion of the spine. Most of their cases, that did not manifest this sign, were of the bulbar type. The next most common sign was muscle tenderness. In all of their cases the sensorium was clear and the patients were coöperative. A few of the bulbar cases were drowsy, but that was apparently due to dehydration and disappeared upon the adequate administration of fluids.

Gordon, Hudson and Harrison[14] tried to produce active immunity, and to demonstrate the effect of passive immunity in monkeys. To establish active immunity they used crude virus, preparations of virus in gelatin containing aluminum and an eluate and found by the neutralization test that there was no evidence to demonstrate that any one of the three antigens was superior or produced a better immunologic response in monkeys. They injected crude virus and a preparation of virus in gelatin containing aluminum into sheep and thought that they obtained a greater antibody response than might be expected with crude virus alone. They were not able to demonstrate any resistance in animals which had been immunized with antigen made of crude virus or with a preparation of virus in gelatin containing aluminum, injected subcutaneously, for the animals became ill when given the disease by the intranasal route. Passive immunity produced by using large amounts of blood from convalescent and hyperimmunized monkeys failed to protect animals when virus was subsequently introduced intranasally. Yet clinical experience fails to reveal more than one attack

in the same individual, perhaps because the virus has remained alive but "imprisoned" in the body.

Many therapeutic agents have been tried in the treatment of poliomyelitis. It is not possible to enumerate all of them. Many have failed on more detailed trials. Among these is potassium chlorate. Contat and his coworkers showed very satisfactory results but Gsell[15] concluded that the clinical basis of the potassium chlorate treatment was unsatisfactory, although his experimental observations were not on a sufficient number of animals to warrant definite conclusions. Saucier and Stewart[30] performed experiments as nearly as possible like those of Contat, Arthus, Spycher and Debat. They failed to protect or influence favorably the course of the disease in monkeys.

Rhett[26] studied the effect of neoprontosil as a prophylactic as well as a therapeutic agent in poliomyelitis. The drug was given to 440 acutely ill children, during an epidemic of poliomyelitis, in approximate dosage of 1 gr. per pound of body weight per day. Of these children only 1 developed paralysis. This was a transient paralysis developing on the fifth day of illness in a child whose mother had not kept up the maintenance dosage of the drug. Symptoms subsided when full dosage was resumed and the paralytic involvement had subsided completely in the 3 weeks' period of quarantine. In 2 other children meningeal symptoms developed on the fifth day of illness and in these also the maintenance dose had not been continued. These symptoms subsided without paralysis with resumption of the full dosage of the drug. He reported also 14 cases of poliomyelitis in which neoprontosil was given in the preparalytic stage. Of these only 1 developed paralysis. In 8 cases, in which a clinical diagnosis of meningeal involvement of poliomyelitis was made, there was subsidence of the condition with neoprontosil medication without paralysis developing. There were 11 cases with paralytic involvement treated during this time by the administration of neoprontosil. Toxic symptoms subsided in this group within 24 to 48 hours, and there was no apparent advance in paralytic involvement after the adequate dosage of the drug was reached and held for 24 to 48 hours. There were no deaths during the acute phase of the disease although there were 2 deaths in the cases included in this series where the condition was fulminating with practically complete involvement of all extremities and respiratory musculature, necessitating the use of the respirator. On the other hand, Toomey and Takacs[35a] concluded from their studies that neither merphenyl borate nor sulfanilamide, in the dosages recommended for clinical use, modifies nor prevents the occurrence of poliomyelitis in monkeys that have been inoculated with the virus of poliomyelitis by way of the intracerebral route. They[35b] found also that sulfapyridine was of no value in preventing the production of experimental poliomyelitis in monkeys inoculated intracerebrally.

Schlutz[31] discussed the chemical substances that have been used intranasally in prophylaxis against poliomyelitis. He stated that among the chemicals which have protective qualities zinc sulphate has been found to be most effective. The mode of action, according to this observer, is not known, but he believed that they do not destroy the virus but rather that they modify the permeability of the tissues which serve as portals of entry. In view of the trend of feeling as regards the

alimentary tract as the means of the virus entering the individual, the use of chemotherapeutic agents in the nasopharynx has no prospect of benefit unless from the nasopharynx it passes into the alimentary system in sufficient quantity to effect blocking of the tissues, if Schlutz's idea of the *modus operandi* of these chemicals as prophylactics is true. This effect of zinc and similar substances has not been substantiated in the observations of recent authors.

Fever therapy has been tried in poliomyelitis. The results have not been promising whatever has been the method of inducing the hyperpyrexia. Kohl[21] used malaria therapy and a protein preparation obtained from bacilli. He did not observe any convincing effects from artificial fever therapy. Rosenow[27] compared the results obtained in the treatment of 221 patients with acute poliomyelitis receiving antistreptococcus serum and the effects of the disease among 116 similar patients living under comparable conditions who did not receive the serum. The initial symptoms were somewhat more severe in the group in which serum was administered than in the control group. The results were contrasted on the basis of mortality and residual paralysis. It was thought that the much lower mortality and lower incidence of residual paralysis of the patients given the serum was directly attributable to the action of the antistreptococcus serum. It was noted that restless, nervous children often fell asleep after the first injection of the serum. Headache and pain in the involved extremity or part were lessened or relieved. The temperature, especially among patients in the preparalytic stages, rapidly fell to normal after an initial transient rise. Progressive paralysis often was apparently arrested. Restoration of muscle function was more rapid among the patients who received the serum.

In summing up a review of the serum therapy and vaccination of poliomyelitis Faber[10b] stated: "It may be that success in preventing poliomyelitis will not come from the methods of classic immunology but from some simpler chemical procedure—whether it is the use of hormones or vitamins or something else—that will modify intracellular metabolism so that the natural host cells will no longer be suitable for invasion and multiplication of virus. The conditions that make them suitable are narrowly limited and delicately balanced. The chemical differences in composition of the anterior horn cells, which are susceptible, and those of many other neurons which are not, cannot be very great, and there is reason to suppose that even in the former the implanted virus dies out in many cases by the process which Pette calls autosterilization, without causing death of the cells. It does not appear to be beyond the range of possibility that physicians will some day know the secret of this process and perhaps learn how to control it."

Meyer[24b] found that when cerebrospinal fluid from persons convalescing from poliomyelitis was mixed with the virus prior to inoculation of monkeys it was effective in protecting them from the infection. He also treated 13 patients with poliomyelitis with cerebrospinal fluid from convalescents with gratifying results. Because the cerebrospinal fluid is easier to obtain and because it is as effective as the serum, if not more so, and because the removal of the cerebrospinal fluid from the donor may be of benefit to him, the author felt that this procedure was of great benefit to all concerned.

Following the use of the mechanical respirator in a case of poliomyelitis that has been widely publicized in the lay press as well as in a number of later cases that gained publicity because of the successful termination of pregnancy in several mothers with respiratory forms of poliomyelitis, this means of maintaining respiratory function in this form of the disease is very well known to the public. During the current epidemic in a number of even small communities in Georgia as well as in some of the larger ones where this equipment was lacking, public subscription has provided the funds to procure respirators. Discussing the use of the respirators Wilson[36] believed that confusion exists about the selection of patients with poliomyelitis for treatment in the respirator and that there is a misconception as to the purpose of respirator treatment. He contended that the respirator will aid only when there is a paralysis of the intercostal muscles or of the diaphragm or in rare instances in which there is hypofunction but not dysfunction of the respiratory centers. Unfortunately, most physicians think of the respirator only as a machine for life saving and for aiding the breathing until enough recovery occurs to enable the patient to carry on by himself. The respirator should be considered a device for providing physiologic rest for the muscles of respiration. Too frequently use of the respirator is delayed too long or the patients are taken out of the machine too soon. Most persons are familiar with the respirator which is generally used. Other new types are being developed. One is a room-sized respirator which is able to take care of several persons at a time. This is reported as giving excellent results. Another type is also being developed. It consists of a rigid or semirigid jacket to be put on the chest and abdomen, making an airtight space over the body. The intermittent evacuation of this space by hand or motor-driven pumps causes respiration to be induced in a manner similar to the Drinker apparatus.

The after-treatment of acute poliomyelitis and more especially the treatment of the residual paralysis come under the province of the orthopedist. During the paralytic stage there is little that can be done except to maintain physiologic rest. Under all possible circumstances the orthopedist should be consulted in order that whatever splinting, counteraction or other measures that will minimize the damage to the affected part can be instituted. Irwin[19] pointed out that the treatment carried out during the acute phase was designed to salvage everything of value from wreckage caused by the devastating invasion of the infecting organism. When there is nothing more to salvage the condition passes into the chronic stage. Here the concern should be of adding whatever surgical procedures are indicated and the fitting of permanent braces.

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GYNECOLOGY AND OBSTETRICS.

UNDER THE CHARGE OF

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UTERINE FIBROIDS.

ALTHOUGH myomatous tumors of the uterus are the most common tumors of the female generative organs, their exact etiology remains unknown. Almost 10 years ago Witherspoon[19] presented evidence to show that the unopposed action of estrin (the ovarian follicular hormone) in the absence of corpus luteum influence is the cause of hyperplasia of the endometrium. He suggested, moreover, that the unopposed action of estrin on the myometrium, if prolonged sufficiently, would result in fibromyomatous growths. This hypothesis was based on a study of 124 cases of fibroids, diagnosed microscopically and of 26 cases of endometrial hyperplasia diagnosed microscopically, in which a second operation was performed for fibroids some 4 years later. In this latter group the associated ovarian and endometrial findings suggested a cause and effect relationship between ovarian follicular cystic formation, hyperplasia of the endometrium and fibromyomata of the uterus. Witherspoon advanced the hypothesis that the unopposed action of estrin on the uterus results in immediate endometrial change characterized by hyperplasia, and secondly, in more latent myometrial changes in the nature of fibromatous growths.

Within the past year Brewer and Jones[3] have presented a study of

the relationship between the corpus luteum and the endometrium in patients with uterine fibroids in which their conclusions are quite at variance with the above generally accepted hypothesis. They remind us that a close relationship is supposed to exist between ovarian function and the development of fibroids because: 1, fibroids develop only during active ovarian life; 2, they usually cease to grow after the menopause or castration; 3, they grow rapidly during pregnancy; 4, sterility in patients with fibroids is thought to be dependent upon the disturbed ovarian function; 5, abnormal uterine bleeding is a common symptom of patients with fibroids and is often considered to be the result of ovarian dysfunction; 6, cystic glandular hyperplasia has been reported frequently in such patients and since this results from excessive or prolonged estrogenic stimulation, estrin has been considered the common etiologic factor; 7, failure of ovulation has been reported by some observers; 8, the frequent finding of multiple follicular cysts in the ovaries has suggested the possibility of excessive estrin production; 9, endometriosis and adenomyosis are frequent concomitant lesions with uterine fibroids and since these lesions occur only during the active life of the ovaries, the importance of the ovarian hormone as a common etiologic agent is assumed. In spite of all this circumstantial evidence in favor of the estrogenic theory, they decided to study the ovarian function and endometrial relationship in 100 unselected and consecutive patients operated upon for uterine fibroids. They found that ovulation had occurred in these patients similar to that in normal women. Forty-six of the patients had corpora lutea of the present cycle and of these the development of the corpora lutea was normal in 41 as evidenced by histologic study not only of the corpora lutea but also of the endometriums which reflected the characteristic responses to normal corpus luteum hormonal stimulation. Small follicular cysts were present in 21 of the 100 patients but in no instance was there evidence that these cysts had had any influence on the normal physiologic processes of the ovaries or the endometriums, none of them showing hyperplasia. Cystic glandular hyperplasia was present in a moderate degree in only 1 instance in the entire 100 cases, which is at marked variance with the generally accepted view.

From the above discussion it may be seen that we still do not know the reason for the development of fibroids. We have, however, gone a long way in the treatment of this condition since hysterectomy was first undertaken with great trepidation. We know that many fibroids need no treatment whatsoever, but merely observation. Of those that require some therapeutic measures, we may select either irradiation, whether by radium or Roentgen ray, or surgical removal either by myomectomy or hysterectomy, and we shall discuss all of these procedures in turn. In the early days of surgery, an operation was often delayed or proscribed because of the so-called myoma heart. It was believed that the myoma had some toxic influence on the heart which increased the risk of operation. In order to test the validity of this theory Jacobs[11] studied, both clinically and electrocardiographically, 30 consecutive patients with proved fibroids, and compared the findings with those of 30 other patients who were operated upon for various pelvic conditions other than fibromyoma. He came to the conclusion that uterine fibromas do not produce any significant changes in the heart.

There was a surprising similarity of symptoms and signs in the two groups. Although some of the myoma group had cardiac damage, he believes that it was not due to the tumor, inasmuch as other exciting disease was present; moreover, similar cardiac damage was present in the control group. No patient died of any cardiac complication despite the presence of coronary artery disease in 3 of them. From this study we may deduce that while the cardiac risk must be taken into account in any operation for myoma, the presence of the myoma, *per se*, does not increase the risk over what it would be for any other major operation.

Irradiation. My former chief, the late Dr. John G. Clark, was one of the American pioneers in the use of radium in the treatment of pelvic disease, and the teachings which he formulated have been followed with slight modification by those who followed him. It will be of interest therefore to review a report by Norris and Behney[15] of 1437 patients treated by radium for benign hemorrhage. We shall concern ourselves with a group of 687 of these cases who had myomas. The important symptom in myoma cases which calls for the use of radium is hemorrhage, but to use an Hibernianism, for those who wish to use radium, the most important thing to know is when not to use it. Norris and Behney list 19 contraindications to the use of radium, which are: 1, When doubt exists as to the accuracy of the diagnosis; 2, the presence of intraperitoneal lesions other than those responsible for the bleeding and which require surgical intervention; 3, rapid growth in the case of a supposed uterine myoma; 4, associated fundal carcinoma; 5, pressure symptoms; 6, softening or other evidence of degeneration; 7, inflammatory lesions within the pelvis, especially those of the adnexa; 8, myoma larger than a 4 months' pregnancy; 9, submucous tumors, especially if pedunculated; 10, myomas in young women; 11, anemia markedly out of proportion to the bleeding; 12, obstructing tumors or malformations that prevent the proper application of radium; 13, radiophobia; 14, the presence of cervical myomas; 15, pregnancy; 16, highly nervous women; 17, previous pelvic operations; 18, painful myomas; 19, myomas after the menopause.

There are good reasons for each one of these contraindications which will occur to the reader with a little reflection, but in some cases exceptions are made; for example, an associated cardiac lesion may make irradiation of a myoma advisable which otherwise would be treated by hysterectomy; or, in an associated fundal carcinoma irradiation may be the method of choice because of extreme obesity or for other special reasons. If it has been decided that the case is a proper one for the use of radium, the dosage should be carefully considered. The efficacy of the remedy in stopping hemorrhage and the ease with which it may be applied constitute definite hazards in this form of therapy, particularly in patients in the child-bearing period. Norris and Behney have made efforts to modify the dosage of radium so that hemorrhage will be checked but without completely sacrificing the ovarian function. Such dosage is less certain and less permanent in its results but they believe that in their earlier cases the dosage employed was too heavy. They found that in patients who received less than 600 mg. hours, the menopausal symptoms were materially less but the results secured were correspondingly diminished. The effect of irradiation in dosages over 600 mg. hours was practically constant both in regard to the percentage

of menopausal symptoms and satisfactory results. Without going into actual figures, their report shows that in dosages over 600 mg. hours, menopausal symptoms will occur in slightly over one-half of the patients and satisfactory results will be achieved in well over 80%. In order to determine the permanency of their results, they followed 184 patients for 10 years or more and only 4 of them (2%) had recurrences. There were 74 cases which failed to get a satisfactory result from one irradiation; of these a second irradiation relieved 37, hysterectomy relieved 30, myomectomy relieved 2, the remaining 5 being unrelieved. There were 3 deaths in the series, 1 from pneumococcic meningitis on the third day, 1 from cerebral hemorrhage on the fifth day and 1 from intestinal obstruction on the tenth day, an operative mortality of 0.4%.

In presenting an analysis of his series of 986 cases of myoma treated by irradiation Costolow[5] states that in his opinion some of the contraindications to irradiation are too rigid and patients for this form of therapy are being selected too conservatively. In his experience preexisting pelvic inflammatory disease is not a justifiable contraindication since acute pelvic inflammation after irradiation occurred in only 1 patient in his series. The average age of his patients was 42.5 years, approximately 75% of the patients being over 40 and therefore, in or near, the menopause. He has found that irradiation menopause is no more troublesome than the normal menopause and furthermore sexual function is not destroyed. In only 4 patients (0.4%) did cancer of the uterus develop after radiation therapy, so that it does not have to be feared in advising such treatment. He states that a remaining uterus is no more likely to become cancerous than a normal uterus in a woman of the cancer age. Inflammatory degeneration of large fibroid tumors occurred in 2 cases, both of which were successfully operated upon at a later date. In tumors the size of a 3 months' pregnancy or smaller (722 cases), there was a reduction of the uterus to normal in 84.3%. Only 2.4% of this group were later operated on. In patients with tumors the size of a 4 months' pregnancy or larger (264 cases) there was a reduction of the uterus to normal in 36.7% and to normal or slightly enlarged (less than twice normal) in 52.7%. Only 4.2% of this group were later operated on. In tumors of this size Roentgen rays alone, preceded by curettage, are the most satisfactory method of radiation therapy in his experience.

In a series of 287 cases of benign uterine tumors Norsworthy[16] found radium to be uniformly satisfactory and while a vaginal discharge persisted for from 3 weeks to several months, it eventually ceased in all cases. The minimum dose he employed was 400 mg. hours; the maximum, 4800 mg. hours, and the series was without mortality.

While of course radium should not be used in the presence of a pregnancy, it is of interest to know what happens if pregnancy should be present or if the patient should become pregnant after the irradiation. Fortunately no one clinic has had an extensive experience with such situations but the literature has been analyzed by Miller, Corscaden and Harrar[14] who find that the opinion is that pregnancies subsequent to irradiation should be divided into "early" and "late." By "early" is meant conception in the first few months after the application of a temporary sterilizing dose of radium. "Late" conception is that which takes place after the end of the temporary amenorrhea. It is believed

that injury is possible in the case of early conception, whereas in late conception the ovum would recover completely before it is capable of being fertilized. It has been noted that there is a tendency toward abortion in the first pregnancies following radiation treatment, whereas later pregnancies are more often normal. It seems likely that in many of the early cases abortion is caused by the disease of the uterus or adnexa for which treatment was instituted or by the effect of the radium on the generative organs rather than as the direct effect upon the fetus. It would seem that physicians using radium should make written records warning the patient and her husband against early subsequent pregnancies and that these warnings should be acknowledged in writing. It also seems advisable to interrupt any pregnancy which has been subjected to therapeutic irradiation, for it is generally admitted that serious effects on the offspring will result in a high percentage of cases. These effects are proportional to the amount of radiation and are more serious in early pregnancies, though the fetus may be seriously injured at any stage of development.

Myomectomy. This operation, when it can be applied, is unquestionably the most conservative as it removes the tumors but leaves a functioning uterus behind. Perhaps the strongest advocate of this procedure is Victor Bonney,[2] of London, who states that women vary in their reactions to hysterectomy; but there is a very definite proportion who take the loss of the womb very much to heart, either because they are aware that to many men the idea of a woman not being "whole" is unattractive, or on account of the irrevocable sterility. Myomectomy has usually had a limited scope, being restricted to cases where the tumors were single, or if multiple, then superficially placed. Resenting this restriction, he has extended the operation until he has been able to perform it in any case if conservation of the uterus is desirable. In a general way his rule is that in patients under 41 years of age the preferential operation is myomectomy, while in patients over that age hysterectomy is desirable, but there are exceptions to the rule. On the one hand is the relatively young woman, who having had sufficient children, wants to have her womb removed, and on the other hand, the elderly woman who, for private reasons, sentimental or otherwise, desires its conservation. He has performed myomectomy 487 times. The fibroids were solitary in 237 cases and multiple in 250 cases. Of the multiple cases, the largest number of tumors he has removed from a single uterus was 125, so it can be seen how far he has extended the indications for the operation. He has had 7 operative deaths (a mortality rate of 1.2%), with no deaths in the last 200 cases. New fibroids have formed in 5% of the cases, but only in 3% to the extent of requiring further operation. All these cases were patients who had developed fibroids at an exceptionally early age and to whom he ascribes a special fibroid-forming tendency. Of 77 women within the child-bearing age who wished and were in a position to have children, 30 (39%) did conceive. Seventy-five per cent of the deliveries were normal and 25% were by Cesarean section. The high percentage of Cesarean operations was due to the relatively advanced age of the mothers and the great obligation to deliver a living child. In speaking of the technique which he employs he lays great emphasis on a special myomectomy clamp which he has invented. This instrument controls both uterine arteries

at once and in addition stops the entire bloodflow from coming up through the cervix. The result is that when in addition the ovarian blood supply is controlled by ring forceps, the circulation through the uterus is completely stopped and the operation becomes bloodless. The incision in the uterus should be central and anterior whenever possible. Tumors situated off the midline can be reached by secondary incisions starting from the main incision and tunneling outward. Tumors lying in the posterior wall if not too large are removed "transcavity," the lumen of the uterus being opened from the front and the posterior wall being incised through the posterior endometrium. He states that there is no risk in opening the uterine cavity and it has the advantage of preventing small submucous fibroids being overlooked. Every fibroid down to the smallest seedling must be removed. A large fibroid in the posterior wall is handled through a transverse incision just behind the uterotubal junction. This incision opens the capsule of the tumor which is then extracted by morcellation after which the remaining posterior uterine wall is brought over the fundus and sutured anteriorly. This leaves a smooth posterior uterine wall opposed to the intestines, whereas if the fibroid is removed through an incision in the posterior wall, a suture line is left directly inviting intestinal adhesion.

In cases in which the number of fibroids to be removed is too great to allow the employment of either of the above methods, he performs a "block excision," bisecting the uterus down nearly to the internal os, removing the tumor area and then reuniting the remaining halves of the uterus. He has also developed a method of removing cervical fibroids by myomectomy, but from the opinions which will be quoted later in this review concerning cervical fibroids, it is doubtful if many gynecologists will adopt his method.

In the opinion of Zondek[20] the technique of myomectomy is much more difficult than that of hysterectomy, to which probably most gynecologists would subscribe. Hysterectomy has a typical technique but myomectomy is atypical; once the abdominal cavity is opened a plan must be made according to the case. It is not sufficient to remove the myomas but in addition it is important to spare the musculature as far as possible in order to preserve a uterus capable of its function in pregnancy and parturition. If the uterus following the operation is a slack, weak organ, it may be capable of menstruation but incapable of carrying a child and he believes that in such cases a high supravaginal amputation is a better procedure. In performing myomectomy he insists that every myoma must be enucleated, the orifice of one tube, preferably both tubes, must be spared, and the uterus must be reconstructed in such a manner as to preserve as much muscle tissue as possible. In order to test the last point he injects an ampule of pitocin into the reconstructed uterus. The reaction to this injection becomes apparent by the contraction and rigidity of the uterus and thus, according to the degree of contraction, the presence or absence of sufficient uterine musculature is demonstrated. He calls attention to the fact that it is possible to ligate the uterine artery on one side without interference with the nutrition of the uterus so that if such ligation becomes necessary in the course of a myomectomy, the surgeon should not assume that hysterectomy must follow. Myomectomy does give a much higher infection rate than hysterectomy because of the large

wound cavities, so that special attention must be paid to asepsis during the operation.

The series of 128 myomectomies reported by Miller and Tyrone[13] represents about 10% of the total number of fibroids treated during the same period at Tulane University in the private service. Where pregnancy was a possibility, about one-third of the cases became pregnant. There was a recurrence of bleeding in 8.5% and only 1 death, an operative mortality of 0.8%. They believe that this low mortality argues very favorably for the safety of myomectomy as compared with hysterectomy, since in this series it was less than one-half that of hysterectomy. They do state, however, that the immediate and remote postoperative complications were of a very serious nature, probably due to faulty operative technique, such as slough of the uterine muscle from tying sutures too tight, or inability to completely cover all raw surfaces. Two cases developed acute intestinal obstruction during their stay in the hospital, both recovering after release of the adhesions which had formed between a loop of ileum and the myomectomy scar. A third patient was 8 months pregnant, following a myomectomy 16 months previously, when the bowel became obstructed in a similar manner, while a fourth patient was operated upon elsewhere for a late obstruction.

After myomectomy at the Mayo Clinic, according to Counseller,[6a] about 35% of the patients became pregnant. He found that recurrences of myomas are more frequent (about 20%) in patients less than 40 and considerably less frequent (8%) in those more than 40 years of age. While he regards the operation as technically more difficult than hysterectomy, the surgical risk is about the same in his experience. A very important point which he mentions is that when a submucous myoma becomes partially or entirely extruded through the cervical canal, the endometrium becomes secondarily infected. It is imperative that these tumors be removed by vaginal myomectomy or approached by vaginal hysterotomy. Should there be associated myomas of the fundus requiring removal, this procedure should be delayed 30 to 60 days until all evidence of infection has subsided.

Hysterectomy. One of the favorite subjects for discussion among gynecologists is the perennial question as to whether a hysterectomy should be complete or subtotal and in a previous review (October, 1937) this question was considered at length. Zondek[20] is of the opinion that there need be no discussion of this subject but that supravaginal hysterectomy should be preferred and performed as high as possible in order to preserve a part of the uterine mucosa from which menstruation may take place. If, however, for technical reasons a high amputation cannot be performed, he states that the uterine mucosa should be implanted in the cervix following a low amputation in order to give the uterus the possibility of menstruating. Even in women near or beyond the menopause he urges the supravaginal operation because the configuration of the vagina is better preserved and difficulties in coitus are eliminated, a fact to which the surgeons pay far too little attention. In the literature one of the reasons often given for performing total extirpation in young women is the opinion that if a stump remains, a cancer of the cervix can occur; he believes that this fear is greatly exaggerated, although he might consider this argument in older women.

He cannot understand why, in younger women, menstruation and the configuration of the vagina should be disturbed for prophylactic reasons only. It would not occur to any surgeon to remove the breast in a woman who had a mammary fibroma because later she might develop carcinoma.

In the large series reported by Danforth[8] total hysterectomy was chosen only in those cases in which the cervix was noticed to be diseased or in which an early coincident cervical malignancy was suspected. He states that subtotal hysterectomy should always be preceded by careful study of the cervix and should only be done when the cervix is quite innocent in appearance. In expert hands the mortality of subtotal and total hysterectomy is about the same, but in the hands of the occasional operator the danger of injury to the bladder and ureters and the greater risk of contamination of the peritoneum increase the risk of operation considerably where total hysterectomy is employed. Therefore, he is of the opinion that frequent use of the more extended operation should not be made by those whose operative experience is small. Should the cervix present evidence of endocervicitis and ectropion, it is possible to do a cervical plastic procedure and a subtotal hysterectomy at the same sitting. By this means a healthy cervix may be left without the necessity of opening into the vagina from above. In cases in which nothing is done to the cervix at the time of operation, later cauterization may be helpful, particularly if some discharge persists. He believes that it is of great importance that at least one ovary be preserved, and that the operation should be carried out in such a way that the ovary will remain in good condition and continue to function. For this purpose it is necessary that a good blood supply be provided and the excision of the uterus carried out in such a manner that the hilum of the ovary with its contained vessels is not disturbed. If the ovary is left mobile the patient will be more comfortable than if it is fixed, therefore he does not attach the ends of the divided broad and round ligaments to the cervix unless it can be done without any tension.

In contrast to the foregoing opinions, in analyzing their series of 1001 cases of fibroids, Baer, Reis and DeCosta[1] state that the incidence of total hysterectomy in their practice has increased from 1.4% to 14.6% over a 10-year period. However, they still employ the supravaginal operation in over one-half of their cases so that they cannot be called enthusiasts over the total operation. Their report also shows that they tend to do fewer myomectomies. The use of radium has steadily diminished, having dropped from 15% to 2%. They remove one or both adnexa in about one-half of the cases. Since they have had a mortality of 0.21% in the last 484 consecutive patients, there can be little doubt but that their judgment and technique have been excellent.

An interesting technical advance has been reported by Wallingford[18] consisting of giving the patient an autotransfusion with blood from a large myoma. After the blood supply to the tumor has been ligated, a needle is inserted into a large uterine vein and the blood aspirated into a citrate solution, filtered and injected into an arm vein, as much as 500 cc. being returned to the patient in this manner. The contraindications to the procedure are infection and malignancy, therefore it is advisable to collect the blood but not inject it until after the uterus has been removed and opened for a careful inspection. If there is any

suggestion of malignancy in the uterus or if there is any infection present in the peritoneal cavity the blood should not be used. This method would be of value only in cases of very large tumors with greatly distended veins.

Uncommon Forms of Myoma. Ordinarily myomata are confined to the body of the uterus. Should hysterectomy be decided upon, the operation is well standardized, and usually presents few difficulties. In some of the uncommon forms of myoma, however, problems arise in their removal which may tax the skill of even the most experienced gynecologist. In considering the management of *intraligamentous myomas*, Counseller[66] refers to the type of tumor which grows out into the cellular tissues of the broad ligament. Since the uterine vessels enter the uterus near the internal os and since the most fixed point in the entire course of the ureter from the kidney to the bladder is closely associated with the tumor at this point, these structures are pushed laterally during the growth of the tumor and may present real difficulties during the operation, frequently resulting in an injury to the ureter. The veins are often enlarged from pressure and under tension, when free from blood, may resemble a ureter. In differentiating between the two, he reminds us that if a ureter is snipped or otherwise irritated with a thumb forceps, it forcibly contracts, distinguishing it from a blood-vessel. Any attempt to remove a large myoma in this location, without adequate exposure and without opening the broad ligament widely so that important structures can be seen will result in troublesome hemorrhage and perhaps injury to the ureter and bladder. It is his custom to open the broad ligament posteriorly, identify and retract the ureter at once so that any troublesome hemorrhage can be immediately controlled without fear of injury to the ureter. When a myoma extends from the posterior wall of the uterus far down behind the peritoneum of the cul-de-sac of Douglas, the ureters are both pushed laterally and the tumor grows upward in the mesentery of the sigmoid colon and the cecum. This type of tumor is often considered inoperable, but it can be removed by careful dissection after retraction of the blood-vessels in the fat of the mesentery of the colon. The safest method, according to Counseller, is to begin the removal by cutting through the peritoneum near the point of origin of the tumor and working laterally, always keeping in intimate contact with the wall of the tumor. A myoma on the lower anterior uterine wall may cause considerable distortion of the bladder. Counseller has found that the posterior wall of the bladder is often intimately attached to the fascial tissues adjacent to the fibroid, rendering difficult the separation of the fibroid from the bladder. If the bladder wall is injured during this separation, he has found it advisable to resect the wall of the bladder, leave it attached to the myoma and close the bladder with 2 rows of plain catgut. This is safer than to run the risk of development of a vesical fistula or secondary hemorrhage from the bladder.

Pedunculated submucous myomas should be removed only by vaginal myomectomy. If the patient has had children, adequate exposure is always possible, but if she is a nullipara it may be necessary to perform a deep lateral episiotomy. The tumor should be grasped with the hand and not with instruments, since such tumors are soft, friable and hemorrhagic. The cervix is usually large and soft enough to permit the index

finger to be introduced along the pedicle to note its union with the uterine wall. It is rarely necessary to ligate the stump, but if bleeding is troublesome the uterus may be packed after the introduction of some antiseptic solution into the uterine cavity.

In considering *cervical fibroids*, Counseller and Collins[7] observe that these tumors commonly affect multiparas, usually at or soon after the menopause. Their first sign is often the appearance of a sudden, large, alarming vaginal hemorrhage. The tumor may fill the pelvis, obstructing all the surrounding organs and often becoming adherent to neighboring structures, making surgical removal very difficult and dangerous. Local removal of either the smaller interstitial or pedunculated subserous types through the vagina may sometimes be done with ease and safety but the large interstitial tumors require an intra-abdominal approach and total hysterectomy. Recently in my own practice I had occasion to remove a very large cervical fibroid by total hysterectomy with all the usual technical difficulties and I was very glad to know that the incidence of these tumors is low, probably around 1%.

In reporting a case of *torsion of a fibromatous uterus*, McIver and Buxton[12] comment on its rarity. The infrequency of this complication is understandable, if one considers the stability of the normal uterus, supported by the broad and round ligaments. These ligaments prevent lateral torsion, though they permit anterior and posterior motion. When the uterus is enlarged and rises out of the pelvis, the cervical segment is elongated, the ligaments are stretched and the chance of torsion becomes greater. The case which McIver and Buxton report occurred in a 62-year-old woman. Acute symptoms and collapse were present. After preoperative transfusion, hysterectomy was performed under local anesthesia and the patient recovered. In these cases the tumor responsible for the twist is usually large, and the changes taking place in the uterus depend upon the extent to which the circulation is cut off; they vary from edema and congestion to actual necrosis. The torsion usually occurs at the isthmus, commonly from left to right and varies in extent from 90 to 450 degrees. These cases fall into two clinical groups: one giving the acute fulminating picture of a strangulated pedicle, while the other presents mild symptoms, without strangulation, which sometimes continue over a period of years. Prompt operation is indicated if the symptoms suggest strangulation.

Fibroids and Pregnancy. According to Charlewood[4] fibroids undergo hypertrophy during pregnancy in response to hormonic influence. Such hypertrophy frequently outstrips the blood supply, and degeneration then occurs. Interstitial fibroids tend to be exteriorized by the contractions and thinning of the uterus during pregnancy. This exteriorization is another factor which tends to interfere with the blood supply and thus cause necrobiosis. The oligemic uterus of the puerperium is responsible for further degenerative changes in fibroids because of the reduced blood supply to the tumors. Moreover, the puerperal uterus grips interstitial and submucous tumors, thereby accentuating the anemic condition of the fibroids. These tumors involute after pregnancy in the same way as normal uterine tissue, sometimes becoming smaller than they were before the pregnancy because of the absorption of at least some of the necrosed tissue.

It is frequently impossible to differentiate on bimanual examination

between a pregnant uterus and one which is symmetrically enlarged due to a soft fibroid. At times even at operation a fibroid uterus is very soft and cystic and has the same color as a pregnant uterus. In case of doubt Greenhill[9] advises studying the relationship of the round ligaments and tubes to the uterine mass. In a myomatous uterus there is usually asymmetry of the round ligaments and tubes, but if they are symmetrical in their relationships to the fundus the surgeon should manipulate the uterus to try to feel a fetus. If this gives no definite information he should inject pituitary extract directly into the uterine musculature. A pregnant uterus will quickly contract symmetrically and become whitish in color. A uterus containing a large fibroid will also contract and become blanched but usually not to the same extent. Furthermore, it may be possible to outline a fibroid distinctly from the main portion of the uterus. If the injection of pituitary extract fails to differentiate between a pregnant and a fibroid uterus, it is safe to cut down into the uterine musculature very gently and deliberately until it is certain that the thick muscle is due to a fibroid or until the bag of waters is seen bulging into the incision. If a fibroid is present, no harm has been done; even when a pregnancy is present, one may cut down to the bag of waters with impunity in most cases. If the incision exposes a pregnancy, it should be sutured and the uterus left intact. If during operation a pregnancy is found in a fibroid uterus, Greenhill believes that some or all of the fibroids should be removed and the gestation left undisturbed. If there are too many fibroids for enucleation, it is best to leave the uterus and the pregnancy alone and close the abdomen. At full term a Cesarean section may be performed and the uterus amputated at the same time.

Reis and Sinykin[17] report 18 myomectomies performed during pregnancy without maternal mortality and with only 2 miscarriages. When performing myomectomy during pregnancy they caution that the uterus be handled as little as possible; the myomectomy should be done with the uterus *in situ*. The uterine cavity should be avoided, as they are convinced that the incidence of postoperative miscarriage rises in direct proportion to the incidence of uterine cavity invasion. Careful hemostasis is necessary to insure a safe outcome. They believe in removal of only the guilty fibroid. In many instances fibroids are multiple, and it is a natural tendency to remove all of them; the authors state, however, that this is a serious mistake, since not only the patient but the pregnancy is much safer if the guilty fibroid alone is removed. They remind us that the majority of fibroids accompanying pregnancy require no treatment. The small group which shows evidence of acute degenerative changes, infection, hemorrhage, those pedunculated fibroids which undergo torsion, those which because of location encroach upon the birth canal, and those which undergo very rapid growth should be removed during pregnancy. The operation should be done at the time indications arise and irrespective of the age of the fetus. Such a procedure obviates the necessity of a Cesarean section followed by myomectomy or hysterectomy, a more complicated procedure than myomectomy followed by vaginal delivery.

When one is confronted with the complication of a fibroid and pregnancy at term Huber and Hesseltine[10] advise that natural delivery should be allowed to go on unless the size, number or location of tumors

produces obstruction to the passage of the fetus. Trial of labor should be permitted in all doubtful cases. When operative interference prior to delivery is indicated by necrosis in the tumor, by obstruction to the birth canal or by failure of normal uterine mechanism, it should consist of Cesarean section with hysterectomy. If operative interference becomes necessary during the puerperium, hysterectomy is also the procedure of choice. They caution that myomectomy with Cesarean section carries a rather high mortality and its employment is generally contraindicated.

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PHYSIOLOGY.

PROCEEDINGS OF

THE PHYSIOLOGICAL SOCIETY OF PHILADELPHIA

SESSION OF OCTOBER 21, 1941

Excitation and Inhibition of Inspiration by Distention of the Lungs. M. G. LARRABEE and G. C. KNOWLTON (Department of Physiology and Biophysics, Cornell Medical College, and Johnson Foundation, University of Pennsylvania). It has recently been shown by Worzniak and Gesell that in addition to the classical Hering-Breuer reflexes of inhibition of inspiration by distention of the lungs and excitation by their collapse, inspiration may also be excited by inflating the lungs. We have analyzed the afferent mechanisms of the two opposing reflexes elicited by lung inflation by recording the impulses discharged over single efferent fibers in the phrenic nerve and single afferent fibers in the vagus nerve of cats.

In order to excite inspiration the lungs must be distended to a volume exceeding the depth reached in normal quiet breathing, whereas very small inflations suffice to inhibit inspiration. When the lungs are rapidly inflated and then held at a volume sufficient to excite inspiration, the efferent discharge which is evoked lasts only about a second,

to be followed by inhibition which is a much more enduring reflex. Both reflexes are eliminated by cutting the vagus nerves.

Records of single afferent fibers show the presence in the lungs of two distinct kinds of stretch receptors, one of which adapts slowly as described by Adrian, while the other kind adapts rapidly when inflation is maintained, usually dropping out of action within a second. The slowly adapting endings are on the average of lower threshold than the rapidly adapting endings, which do not come into action during normal quiet breathing.

Therefore the two kinds of response to inflation of the lungs may be explained by assuming that impulses from the slowly adapting receptors inhibit inspiration and that impulses from the rapidly adapting receptors excite inspiration. This interpretation of afferent function also accounts for excitation of inspiration by forced deflation of the lungs, for such deflation reduces the activity of many slowly adapting receptors, and many of the rapidly adapting receptors are stimulated by deflation as well as by inflation.

It is suggested that the rapidly adapting receptors are part of an autogenous pulmonary reflex mechanism, which increases the depth of any unusually deep inspiration, and permits very deep inspirations to be caused by relatively weak stimuli from other parts of the body.

Rôle of the Kidney in the Maintenance of Arterial Blood Pressure in Hemorrhage.* ANGIE S. HAMILTON and DEAN A. COLLINS (Department of Physiology, Temple University, School of Medicine). Since the kidney increases its production of pressor material in experimental renal hypertension, it appeared possible that this phenomenon might have a physiologic counterpart in conditions of stress altering renal circulation. To test this thesis for severe hemorrhage, three types of experiments were performed on over 50 dogs anesthetized with chloralose.

1. Animals without renal circulation were unable to maintain their blood pressures in hemorrhage as well as controls subjected to corresponding sham procedures. Thus a blood loss of 38 cc. per kg. in 50 minutes lowered the blood pressures of the two groups to averages of 23 and 63 mm. Hg respectively. Half of the animals without renal circulation died shortly after the conclusion of the hemorrhage. Since other known renal functions cannot explain these differences, they suggest the liberation of pressor material by the kidney.

2. Suggestive evidence was obtained that after extreme hemorrhage animals with intact renal circulation are partially or completely tachyphylactic to injected renin, while nephrectomized dogs are not.

3. Samples of blood from the renal vein and femoral artery were tested for pressor activity by injection into a nephrectomized animal. Both bloods collected before hemorrhage gave insignificant responses. After hemorrhage (34–45 cc. per kg.) renal vein and femoral artery bloods gave pressor responses of 15–28 and 9–13 mm. Hg respectively.

The above results support the thesis that the kidney liberates pressor material in hemorrhage and that this process constitutes a compensatory mechanism. This mechanism may be involved in hemorrhagic shock.

* Supported by a grant from the American Philosophical Society.

The Rôle of Carbonic Anhydrase in Certain Ionic Exchanges. M. H. JACOBS and DOROTHY R. STEWART (Departments of Physiology, University of Pennsylvania and Skidmore College). One of the authors (Cold Spring Harbor Symposia, **8**, 30, 1940) has suggested a mechanism, analogous to chemical catalysis but involving a diffusion equilibrium rather than a chemical equilibrium, by which the rate of entrance of certain ammonium salts into the erythrocyte is greatly accelerated by low concentrations of bicarbonates (Ørskov: Pflüger's Arch., **231**, 680, 1933). An essential part of the suggested mechanism is a cycle involving the conversion within the cell of CO_2 to HCO'_3 and the reverse change in the surrounding solution. A necessary consequence of such a theory would be an influence on the over-all rate of the process of the enzyme carbonic anhydrase. Such an influence, very striking in its magnitude, has been found, both by inactivating the carbonic anhydrase already present in the cells with KCN or sulfanilamide, and also by the addition under appropriate conditions of more of the enzyme to the surrounding solution. With concentrations of sulfanilamide approaching saturation, the bicarbonate effect may be almost completely (but reversibly) abolished without greatly altering the general osmotic properties and permeability of the cell. Under properly chosen conditions a measurable inhibition can be demonstrated with concentrations of sulfanilamide as low as a few hundredths of a milligram per 100 cc. Sulfapyridine seems completely to lack this inhibitory power. By the same general methods the principle of catalysis of diffusion by bicarbonates can be extended to the volume changes of erythrocytes which occur on altering the pH of the medium, and also to exchanges of Cl' from the erythrocyte for SO''_4 from the surrounding medium. There is reason to believe that bicarbonates and carbonic anhydrase may also behave in a similar manner in certain tissues other than the blood.

The Depression of Metabolism During Diving. LAURENCE IRVING, P. F. SCHOLANDER and S. W. GRINNELL (Edward Martin Laboratory, Swarthmore College). It has been shown that the use of oxygen by seals during recovery from a dive frequently fails to compensate for the lack of oxygen use during the dive (Scholander, P. F., Hvalraadets Skrifter, No. 22, Det Norske Videnskaps Akademi i Oslo, pp. 1–129, 1940). A decline of body temperature has also been observed during a dive, and the two observations support the view that the level of metabolism of the seal is depressed during diving.

The possibility that the metabolic rate of a mammal can thus be quickly depressed was tested in studies of the three-toed sloth, *Bradypus tridactylus*, at Barro Colorado Island, Panama Canal Zone. The sloths easily endured repeated submersions for 20 minutes. Measurements of respiratory metabolism were recorded by Scholander's difference respirometer. In recovery from dives, there was very little or no extra use of oxygen to indicate a restitution of any considerable anaërobic process. The small lactic acid formation in the dive had practically disappeared after 10 minutes recovery. It is thus shown that the metabolism of the sloth is very much depressed during a dive. The reduction conserves oxygen stores and is of regular occurrence in seals.

In a natural diver its regular usefulness in conserving oxygen is apparent. In a sloth the emergency situation is unusual, but it shows that it is possible for the level of metabolism to be depressed quickly in respiratory stress.

Correction. In the article by Coggeshall, Bennett, Warren and Bauer on "Synovial Fluid and Synovial Membrane Abnormalities . . . ." in the October issue of this Journal (p. 486) the last word in the last sentence above Chart 3 on p. 490 should read "abnormal," instead of "normal." It should be noted also that Dr. Bennett's position in the School is Assistant Professor of Pathology, instead of Assistant Professor in Medicine.

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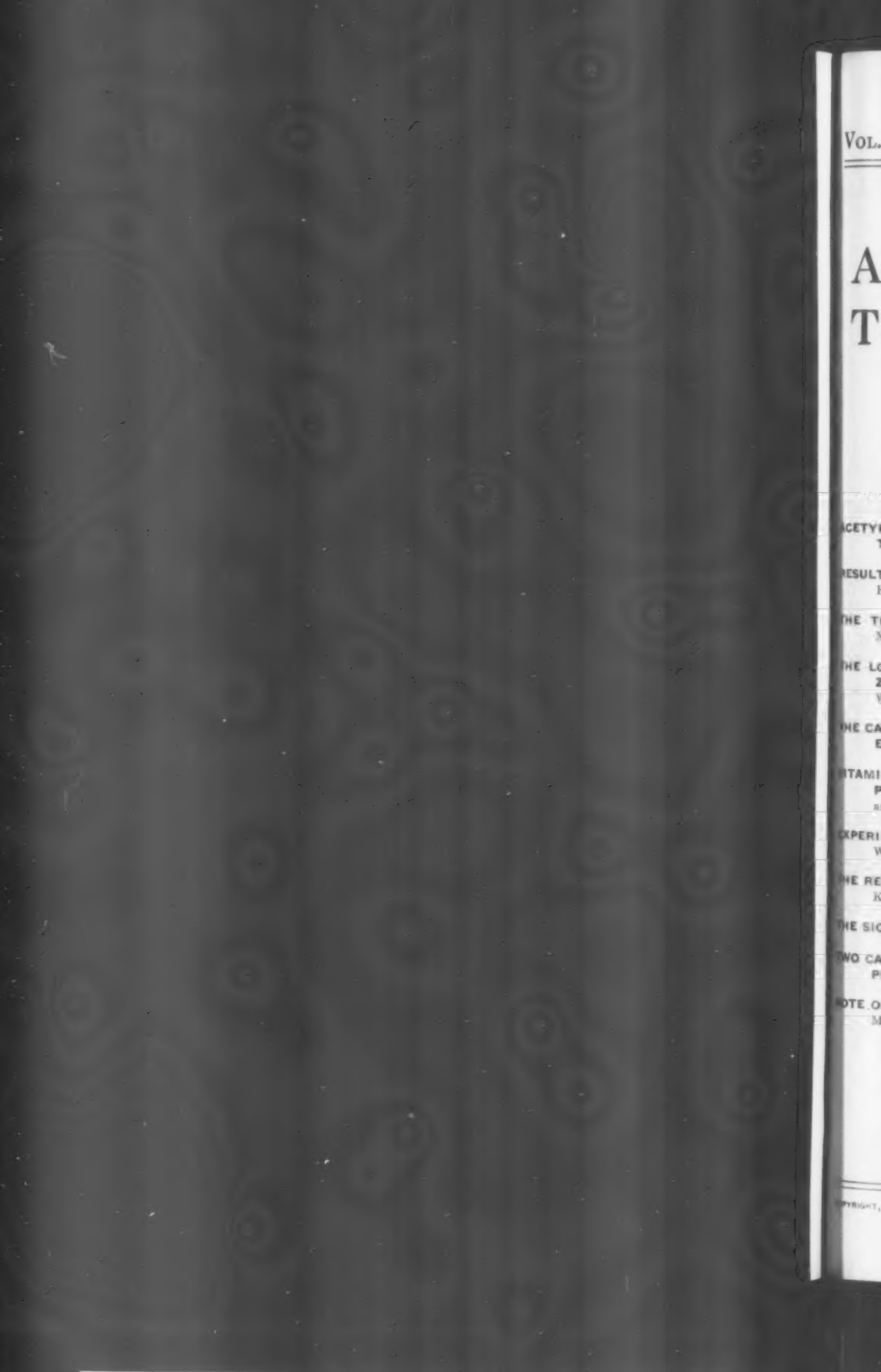

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VOL. 202 No. 6 DECEMBER, 1941 No. 837

JUN 25 1942

THE AMERICAN JOURNAL OF THE MEDICAL SCIENCES

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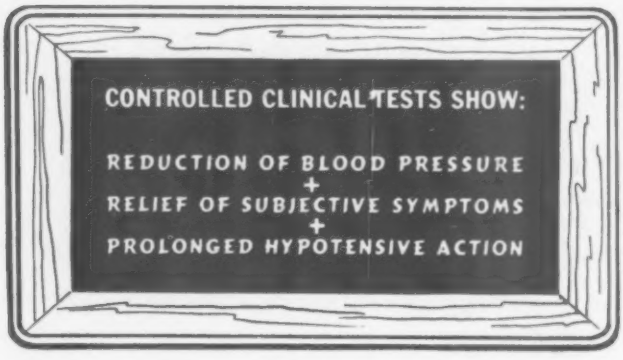

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* Fisher and Apperly: J. Lab. Clin. Med. 26: 823-27, 1941. Reprints available upon request.

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Are the Neuritic Symptoms of Pregnancy *due to a deficiency of vitamin B1 (thiamine)?*

SUCH common neuritic symptoms of pregnancy as pains in arms and legs, muscle weakness, and (less frequent but more serious) paralysis of the extremities may result from a shortage of antineuritic vitamins, recent investigations appear to show. Although neuronitis of pregnancy has long been considered a toxemia, no toxins have ever been identified.

Clinical observations of Strauss and McDonald lead to the conclusion that the condition is a dietary deficiency disorder similar to beriberi, caused by lack of vitamin B_1. They report recovery in their cases receiving this therapy, including dried brewers' yeast.

Hyperemesis as Cause of Avitaminosis

Wechsler observes that all cases of polyneuritis of pregnancy recorded in the literature were preceded by long periods of severe vomiting. "It would seem," he adds, "that because of actual starvation these patients suffered from avitaminosis and consequent neuritis," a view likewise held by Hirst, Luikart, and Gustafson. Plass and Mengert observe that the practice of giving high carbohydrate feedings for hyperemesis gravidarum is still more likely to cause avitaminosis.

Dried brewers' yeast, as it is far richer than any other food in vitamin B_1 (thiamine), is being used with benefit both in the prevention and treatment of polyneuritic symptoms of pregnancy. Lewy found that additions of yeast to the diet reduced electric irritability of the peripheral nerves and brought clinical improvement. Vorhaus states that he and his associates, after administering large amounts of vitamin B_1 (thiamine) to 250 patients having various types of neuritis, including that of pregnancy, observed in about 90% of cases "varying degrees of improvement, i.e., from partial relief of pain to complete disappearance of all symptoms."

Need for Vitamin B_1 (thiamine) in Lactation

Evans and Burr, Hartwell, Sure and co-workers, and Macy *et al* are among numerous authorities who find that the nursing mother also needs a supplement of vitamin B_1 (thiamine) from 3 to 5 times the normal requirement. It is accepted that during pregnancy and lactation the requirement for vitamin G (riboflavin) is increased.

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